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(54) Title: FUNGAL PROMOTERS ACTIVE IN THE PRESENCE OF GLUCOSE

(57) Abstract

A method is described for the identification and cloning of promoters that express under a defined environmental condition, such as growth in glucose medium. Using this method, five *Trichodermal* promoters capable of the high expression of operably linked coding sequences are identified, one of which is the promoter for *T. reesei tef1*. Also provided are altered *cbh1* promoters, altered so that glucose no longer represses expression from such promoter. The invention further provides vectors and hosts that utilize such promoters, and unique fungal enzyme compositions from such hosts.

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Title of the Invention

Fungal Promoters Active in the Presence of Glucose

Cross-Reference to Related Applications

This application is a continuation-in-part of U.S. Application No. 07/496,155 filed March 19, 1990.

Background of the Invention

I. Methods for the Identification of Promoters

Many systems have been used to isolate genes and their promoters located immediately upstream of the translation start site of a gene. The techniques can roughly be divided in two categories, namely (1) where the aim is to isolate genomic DNA fragments containing promoter activity randomly by so-called promoter probe vector systems and (2) where the aim is to isolate a gene *per se* from a genomic bank (library) and isolation of the corresponding promoter follows therefrom.

In promoter probe vector systems, genomic DNA fragments are randomly cloned in front of the coding sequence of a reporter gene that is expressed only when the cloned fragment contains promoter activity (Neve, R.L. *et al.*, *Nature 277*:324-325 (1979)). Promoter probe vectors have been designed for cloning of promoters in *E. coli* (An, G. *et al.*, *J. Bact. 140*:400-407 (1979)) and other bacterial hosts (Band, L. *et al.*, *Gene 26*:313-315 (1983); Achen, M.G., *Gene 45*:45-49 (1986)), yeast (Goodey, A.R. *et al.*, *Mol. Gen. Genet. 204*:505-511 (1986)) and mammalian cells (Pater, M.M. *et al.*, *J. Mol. App. Gen. 2*:363-371 (1984)). Because it is well known in the art that *Trichoderma* promoters fail to work in *E. coli* and yeast (e.g. Penttilä, M.E. *et al.*, *Mol. Gen. Genet. 194*:494-499 (1984)), these organisms cannot be used as hosts to isolate *Trichoderma* promoters. Due to the fact that,

during the transformation of *Trichoderma*, the transforming DNA integrates into the fungal genome in varying copies in random locations, application of this method by using *Trichoderma* itself as a cloning host is also unlikely to succeed and would not be practical for efficient isolation of *Trichoderma* promoters with the desired properties.

Known genes can be isolated from either a cDNA or chromosomal gene bank (library) using hybridization as a detection method. Such hybridization may be with a corresponding, homologous gene from another organism (e.g., Vanhanen *et al.*, *Curr. Genet. 15*:181-186 (1989)) or with a probe designed on the basis of expected similarities in amino acid sequence. If amino acid sequence is available for the corresponding protein, an oligonucleotide can also be designed which can be used in hybridization for isolation of the gene. If the gene is cloned into an expression bank, the expression product of gene can be also detected from such expression bank by using specific antibodies or an activity test.

Specific genes can be isolated by using complementation of mutations in *E. coli* or yeast (e.g., Keesey, J.K. *et al.*, *J. Bact. 152*:954-958 (1982); Kaslow, D.C., *J. Biol. Chem. 265*:12337-12341 (1990); Kronstad, J.W., *Gene 79*:97-106 (1989)), or complementation of corresponding mutants of filamentous fungi for instance by using SIB selection (Akins *et al.*, *Mol. Cell. Biol. 5*:2272-2278 (1985)).

However, a major concern is how to isolate specific genes that have the desired promoter properties, for example genes which would be most highly expressed when glucose is present in the medium. There is no information available in literature to indicate which genes are the most highly expressed in an organism, and especially not from filamentous fungi. The *phosphoglyceratekinase* (*PGK*) promoter from the yeast *Saccharomyces cerevisiae* is considered to be a strong promoter for protein production. However, results obtained by the inventors have shown that the corresponding *Trichoderma* promoter is not suitable for such protein production. Thus, the identification of specific *Trichoderma* genes for their isolation in order to

obtain the best possible promoter for protein production in certain desired conditions is unknown and cannot be predicted. Consequently one cannot rely on any previous nucleotide or amino acid sequence information, nor complement any previously known mutations, in gene isolation for such purpose in *Trichoderma*.

Differential hybridization has been used for cloning of genes expressed under certain conditions. The method relies on the screening of a bank separately with an induced and noninduced cDNA probe. By this method e.g., *Trichoderma reesei* genes strongly expressed during production of cellulolytic enzymes have been isolated (Teeri, T. *et al.*, *Bio/Technology 1*:696-699 (1983)). The differential hybridization methods used are based on the idea that the genes searched for are expressed in certain conditions (like cellulases on cellulose) but not in some other conditions (like cellulases on glucose) which enables picking up clones hybridizing with only one of the cDNA probes used. However, for isolation of the genes expressed strongly on glucose, this approach (expression on glucose and not on some other media) is not a suitable one, and might in fact result in not finding the most highly expressed genes. This is because when differentially screening a chromosomal bank, only induced genes are selected. Such induced genes are not necessarily the most strongly expressed genes. Thus, no method is known in the art which would permit the identification of promoters which function strongly in *Trichoderma* on glucose medium.

Another option for obtaining a promoter with desired properties is to modify the already existing ones. This is based on the fact that the function of a promoter is dependent on the interplay of regulatory proteins which bind to specific, discrete nucleotide sequences in the promoter, termed motifs. Such interplay subsequently affects the general transcription machinery and regulates transcription efficiency. These proteins are positive regulators or negative regulators (repressors), and one protein can have a dual role depending on the context (Johnson, P.F. and McKnight, S.L. *Annu. Rev. Biochem. 58*:799-839 (1989)). However, even a basic understanding of the

regions responsible for regulation of a promoter requires a considerable amount of experimental data, and data obtained from the corresponding promoter of another organism is usually not useful (see Vanhanen, S. *et al.*, *Gene 106*:129-133 (1991)), or at least not sufficient, to explain the function of a promoter originating from another organism.

II. Translation Elongation Factors

Translation Elongation Factors (TEFs) are universally conserved proteins that promote the GTP-dependent binding of an aminoacyl-tRNA to ribosomal A-site in protein synthesis. Especially conserved is the N-terminus of the protein containing the GTP binding domain. TEFs are known as very abundant proteins in cells comprising about 4-6% of total soluble proteins (Miyajima, I. *et al.*, *J. Biochem. 83*:453-462 (1978); Thiele, D. *et al.*, *J. Biol. Chem. 260*:3084-3089 (1985)).

tef genes have been isolated from several organisms. In some of them they constitute a multigene family. Also a number of pseudogenes have been isolated from some organisms. The promoter of the human *tef* gene can direct transcription *in vitro* at least 2-fold more effectively than the adenovirus major late promoter, which indicates that the *tef* promoter is a strong promoter in mammalian expression systems (Uetsuki *et al.*, *J. Biol. Chem. 264*:5791-5798 (1989)). Both the human and the *A. thaliana tef1* promoter (for translation elongation factor EF-1α) has been used in an expression system with high efficiency of gene expression (Kim *et al.*, *Gene 91*:217-223 (1990); Curie *et al.*, *Nucl. Acid Res. 19*:1305-1310 (1991)). In both cases the full expression of the promoter was dependent on the presence of the intron in the 5′ noncoding region.

tef is quite constitutively expressed, the major exception being its expression in aging and quiescent cells. It is not known to be regulated by the growth substrates of the host.

III. Expression of Recombinant Proteins in Trichoderma

The filamentous fungus *Trichoderma reesei* is an efficient producer of hydrolases, especially of different cellulose degrading enzymes. Due to its excellent capacity for protein secretion and developed methods for industrial cultivations, *Trichoderma* is a powerful host for production of heterologous, recombinant proteins in large scale. The efficient production of both homologous and heterologous proteins in fungi relies on fungal promoters. The promoter of the main cellulase gene of *Trichoderma*, cellobiohydrolase 1 (*cbh1*), has been used for production of heterologous proteins in *Trichoderma* grown on media containing cellulose or its derivatives (Harkki *et al.*, *Bio/Technology 7*:596-603 (1989); Saloheimo *et al.*, *Bio/Technology 9*:987-990 (1991)). The *cbh1* promoter cannot be used when the *Trichoderma* are grown on glucose containing media due to glucose repression of *cbh1* promoter activity. This regulation occurs at the transcriptional level and thus glucose repression could be mediated through the promoter sequences. It is also known that cellulase genes *cbh1*, *cbh2*, *egl1* and *egl2* are coexpressed in various growth conditions, thus it is presumable that same regulatory factors operate on fairly similar promoter sequences mediating similar functions. However, nothing is yet known of the mechanism of glucose repression at the promoter level in filamentous fungi.

Glucose repression in the yeast *Saccharomyces cerevisiae* has been studied for many years. These studies have however failed, until recently, to identify binding sequences in promoters or regulatory proteins binding to promoters which would mediate glucose repression. The first ever published glucose repressor protein and the binding sequence in eukaryotic cells was published by Nehlin and Ronne (Nehlin, J.O. and Ronne, H. *EMBO J.* 9:2891-2899 (1990)). This MIG1 protein seems to be responsible of one fifth of the glucose repression of *GAL* genes in *Saccharomyces cerevisiae*, other factors still being required to obtain full glucose repression effect (Nehlin, J.O. *et al.*, *EMBO J. 10*:3373-3377 (1991)).

Thus, it is desirable to be able to produce proteins in *Trichoderma* grown on glucose. Not only is the substrate glucose cheap and readily available, but also *Trichoderma* produces less protease activity when grown on glucose. Further, cellulase production is repressed when *Trichoderma* is grown on glucose, thus allowing for the easier purification of the desired product from the *Trichoderma* medium. Nevertheless, to date there has been no identification or characterization of any promoter that is highly functional in *Trichoderma* grown on glucose. In addition, no modifications of the normally glucose repressed promoter, the *cbh1* promoter, have been identified which would allow the use of this strong promoter for expression of heterologous genes in *Trichoderma* grown on glucose.

Summary of the Invention

This invention is first directed to the identification of the motif, the DNA element, that imparts glucose repression onto the *Trichoderma cbh1* promoter.

The invention is further directed to a modified *Trichoderma cbh1* promoter, such modified promoter lacking such glucose repression element and such modified promoter being useful for the production of proteins, including cellulases, when the host is grown on glucose medium.

The invention is further directed to a method for the isolation of genes that are highly expressed on glucose, especially from filamentous fungal hosts such as *Trichoderma*.

The invention is further directed to five such previously undescribed genes and their promoters from *Trichoderma reesei*.

The invention is further directed to specific cloning vectors for *Trichoderma* containing the above mentioned sequences.

The invention is further directed to filamentous fungal strains transformed with said vectors, which strains thus are able to produce proteins such as cellulases on glucose.

The invention is further directed to a process for producing cellulases or other useful enzymes on glucose.

Brief Description of the Drawings

Figure 1 shows the plasmid pTHN1 which carries the *tef1* promoter and 5' part of the coding region and shows the relevant features of the *tef1* gene and the sequenced areas. Figure 1A is the nucleotide sequence of the *tef1* promoter and coding sequence [TEF001; SEQ ID 1]. The promoter sequence stops at base number 1234. The methionine codon of the start site of translation is located at base numbers 1235-1237 and is underlined. The total number of bases shown is 3461. The DNA sequence composition is 850A, 1044C, 860G, 697T, and 10 other.

Figure 2 shows the plasmid pEA33 which carries the *tef1* promoter and the coding region with relevant features.

Figure 3 shows the plasmid pTHN3 which carries the promoter and coding region of the clone cDNA1 and shows the relevant features. Figure 3A is the nucleotide sequence of the cDNA1 promoter and coding sequence [SEQ ID 2]. The promoter sequence stops at base number 1157. The methionine codon of the start site of translation is located at base numbers 1158-1160 as numbered in Figure 3A and is underlined.

Figure 4 shows the plasmid pEA10 which carries the promoter and coding region of the clone cDNA10 and the relevant regions and sequenced areas. Diagonally hatched = insert; solid line = sequenced region (genomic DNA); squared criss-crossed = sequenced region (cDNA). Not all *Eco*RV and *Nde*I sites are shown. Figure 4A is the nucleotide sequence of the cDNA10 promoter and coding sequence [CDNA10SEQ; SEQ ID 3]. The promoter sequence stops at base number 1522. The methionine codon of the start site of translation is located at base numbers 1523-1525 and is underlined. The total number of bases shown is 2868. The DNA sequence composition is 760A, 765C, 675G and 668T.

Figure 5 shows the plasmid pEA12 which carries the clone cDNA12 and relevant features and sequenced areas. Diagonally hatched = insert; solid line = sequenced region (genomic DNA); squared criss-crossed = sequenced region (cDNA). ? = unsequenced intron region. Note: *Aval* is not a unique site. Figure 5A is the nucleotide sequence of the cDNA12 promoter and coding sequence [A12DNA; SEQ ID 4]. The promoter sequence stops at base number 1101. The methionine codon of the start site of translation is located at base numbers 1102-1104 and is underlined. The total number of bases is 2175. The DNA sequence composition is 569A, 602C, 480G, 519T and 5 other.

Figure 6 shows the plasmid pEA155 which carries the promoter and coding region of the clone cDNA15 and the relevant features and sequenced areas. Diagonally hatched = insert; solid line = sequenced region (genomic DNA); squared criss-crossed = sequenced region (cDNA). Not all *Pst*I and *Eco*RI sites are shown. Figure 6A is the nucleotide sequence of the cDNA15 promoter and coding sequence [SEQ ID 5]. The total number of bases is 2737. The DNA composition is 647A, 695C, 742G, 649T and 4 other.

Figure 7 shows plasmid pPLE3 which carries the *egl1* cDNA. Just above the plasmid map is the sequence of the adaptor molecule [SEQ ID 25] that was constructed to remove the small *Sac*II and Asp718 fragment from the plasmid so as to construct an exact joint [SEQ ID 26, SEQ ID 27] between the *cbh1* promoter and the *egl1* signal sequences [SEQ IDs 18 and 16]. Figure 7A shows the 1588 bp sequence of the *egl1* cDNA (369A, 527C, 418G and 274T) [SEQ ID 16]. Figure 7B shows the sequence of the 745 bp *cbh1* terminator of pPLE131 (198A, 191C, 177G, and 179T) [SEQ ID 23].

Figure 8 shows construction of plasmid pEM-3A and SEQ ID 28. The "A" on the plasmid maps denotes the EGI tail sequence and the "B" denotes the EGI hinge sequence.

Figure 9 shows the plasmid pTHN100B for expression of the EGIcore under the *tef1* promoter and SEQ ID 28.

Figure 10 shows production of EGIcore from the plasmid pTHN100B into the culture medium of the host strain QM9414 analyzed by EGI specific antibodies from a slot blot. Lane 1: pTHN100B-16b, 200 μl glucose supernatant; lane 2: QM9414, 200 μl glucose supernatant; lane 3: TBS; lane 4: QM9414, 200 μl solka floc 1:500 diluted supernatant; lane 5: QM9414, 200 μl solka floc 1:5,000 diluted supernatant; lane 6: QM9414, 200 μl solka floc 1:10,000 diluted supernatant; lane 7: pTHN100B-16b, 200 μl glucose 1:5 diluted supernatant; lane 8: QM9414, 200 μl glucose 1:5 diluted supernatant; lane 9: 200 ng EGI protein; lane 10: 100 ng EGI protein; lane 11: 50 ng EGI protein; and lane 12: 25 ng EGI protein.

Figure 11 shows Western blotting with EGI specific antibodies of culture medium of the strain pTHN100B-16c grown in whey-spent grain or glucose medium, and of EGIcore purified from the glucose medium. Lane 1: pTNH100B-16c, 10 μl whey spent grain supernatant; lane 2: pTNH100B-16c, 5 μl whey spent grain supernatant; lanes 3-5: EGIcore purified from pTHN100B-16c glucose fermentation; lane 6: pTHN100B-16c, 15 μl glucose fermenter supernatant, concentrated 100x; lane 7: pTHN100B-16c, 7.5 μl glucose fermenter supernatant, concentrated 100x; and lane 8: low molecular weight markers at 94kDa, 67 kDa, 43 kDa, 30 kDa and 20.1 kDa (bands 1-5 starting from lane 8, top of gel).

Figure 12 shows Western blotting of culture medium of the strain pTHN100B-16c grown on glucose medium. Lane 1: EGI protein, about 540 ng; lane 2, EGI protein, about 220 ng; lane 3, EGI protein, about 110 ng; lane 4: pTHN100B-16c, 30 μl glucose fermenter supernatant; lane 5: pTHN100B-16c, 30 μl glucose fermenter supernatant, concentrated 4.2x; lane 6: low molecular weight markers at 94kDa, 67 kDa, 43 kDa, 30 kDa and 20.1 kDa (bands 1-5 starting from lane 6, top of gel).

Figure 13 diagrams the elements of the plasmid pMLO16. Figure 13A is the sequence of the *cbh1* promoter of plasmid pML016 [SEQ ID18]. Figure 13B is the sequence of the *T. reesei cbh1* terminator on plasmid pML016 and plasmids derived from it [SEQ ID24].

Figure 14 shows the expression of β-galactosidase on glucose medium in pMLO16del5(11)-transformants of *Trichoderma reesei* QM 9414 (A2-F5). A1: QM 9414 host strain; C1 and E1: QM 9414 transformant in which one copy of β-galactosidase expression cassette with intact *cbh1* promoter has replaced the *cbh1* locus; B1, D1 and F1: empty wells.

Figure 15 shows the restriction map of the plasmid pMLO16del5(11), which carries the shortened form of the *cbh1* promoter fused to the *lacZ* gene and the *cbh1* terminator. Figure 15A is the sequence of the truncated *cbh1* promoter [(pMLO16del5(11)); SEQ ID19]. The polylinker is underlined. The arrow denotes the deletion site.

Figure 16 shows the restriction map of the plasmid pMLO17, which carries the shortened form of the *cbh1* promoter fused to the *cbh1* chromosomal gene. The restriction sites marked with a superscripted cross "+" are not single sites. There are two additional *Eco*RI sites in the *cbh1* gene that are not shown. Figure 16A shows the sequence of the *Ksp*I-*Xma*I fragment (the underlined portion) that contains the chromosomal *cbh1* gene [SEQ ID17].

Figure 17 shows the expression of CBHI on glucose medium in pMLO17 transformants of *Trichoderma reesei* QM 9414. A collection of single spore cultures (number and a letter-code) and different control samples are shown.

Figure 18 shows specific mutations of mig-like sequences (M) in *cbh1* promoters of pMI-24, pMI-25, pMI-26, pMI-27 and pMI-28. The promoters shown here were fused to *lacZ* gene and *cbh1* terminator as described for pMLO16 (see Figure 13) or pMLO16del0(2) (see Figure 19). *: sequence alteration made in *cbh1* promoter in different combinations. At position -1505-1500 the genomic sequence is 5'-CTGGGG and the altered sequence is 5'-TCTAAA. At position -1001-996 the genomic sequence is 5'-CTGGGG and the altered sequence is 5'-TCTAAA. At position -720-715 the genomic sequence is 5'-GTGGGG and the altered sequence is 5'-TCTAGA. pMLO16del0(2) was used as a starting vector for pMI-25, pMI-26, pMI-27

and pMI-28, pMLO16 for pMI-24. v = the polylinker. Figure 18A is the sequence of the altered *cbh1* promoter of pMI-24 (PMI27PROM) ([SEQ ID20]). The total number of bases is 1776. The sequence composition is 487A, 399C, 434G, and 456T. The polylinker is underlined and the sequence alteration is boxed. Figure 18B is the sequence of the altered *cbh1* promoter of pMI-27 ([SEQ ID21]). The polylinker is underlined, the arrow denotes the deletion point and the sequence alterations are boxed. Figure 18C is the sequence of the altered *cbh1* promoter of pMI-28 (PMI28PROM) ([SEQ ID22]). The polylinker is underlined, the arrow denotes the deletion point and the sequence alterations are boxed. The total number of bases is 1776. The sequence composition if 490A, 399C, 430G and 457T.

Figure 19 shows the restriction map of the plasmid pMLO16del0(2), which carries the shortened form of the *cbh1* promoter fused to *lacZ* gene and the *cbh1* terminator.

Figure 20 shows the expression of β-galactosidase on indicated medium in *Trichoderma reesei* QM9414 transformed with pMLO16del0(2), pMI-25, pMI-27, pMI-28, pMLO16 and pMI-24.

Detailed Description of the Preferred Embodiments

I. Identification of Fungal Genes that Express on Glucose Medium

In the following description, reference will be made to various methodologies known to those of skill in the art of molecular genetics and biology. Publications and other materials setting forth such known methodologies to which reference is made are incorporated herein by reference in their entireties as though set forth in full.

General principles of the biochemistry and molecular biology of the filamentous fungi are set forth, for example, in Finkelstein, D.B. *et al.*, eds., *Biotechnology of Filamentous Fungi: Technology and Products*, Butterworth-Heinemann, publishers, Stoneham, MA (1992) and Bennett, J.W. *et al.*, *More*

Gene Manipulations in Fungi, Academic Press - Harcourt Brace Jovanovich, publishers, San Diego CA (1991).

To be able to develop versatile systems for protein production from *Trichoderma*, especially when *Trichoderma* are grown on glucose, a method has been developed for the isolation of previously unknown *Trichoderma* genes which are highly expressed on glucose, and their promoters. The method of the invention requires the use of only one cDNA population of probes.

It is to be understood that the method of the invention would be useful for the identification of promoter sequences that are active under any desired environmental condition to which a cell could be exposed, and not just to the exemplified isolation of promoters that are capable of expression in glucose medium. By "environmental condition" is meant the presence of a physical or chemical agent, such agent being present in the cellular environment, either extracellularly or intracellularly. Physical agent would include, for example, certain growth temperatures, especially a high or low temperature. Chemical agents would include any compound or mixtures including carbon growth substrates, drugs, atmospheric gases, etc.

According to the method of the invention, the organism is first grown under the desired growth condition, such as the use of glucose as a carbon source. Total mRNA is then extracted from the organism and preferably purified through at least a polyA+ enrichment of the mRNA from the total RNA population. A cDNA bank is made from this total mRNA population using reverse transcriptase and the cDNA population cloned into any appropriate vector, such as the commercially available lambda-ZAP vector system (Stratagene). When using the lambda-ZAP vector system, or any lambda vector system, the cDNA is packaged such that it is suitable for infection of any *E. coli* strain susceptable to lambda bacteriophage infection.

The cDNA bank is transferred by standard colony hybridization techniques onto nitrocellulose filters for screening. The bank is plated and plaque lifts are taken onto nitrocellulose. The bank is screened with a population of labelled cDNAs that had been synthesized against the same RNA

population from which the cloned cDNA bank was constructed, using stringent hybridization conditions. It should be noted that the genes are not expressed in any way during this selection process. This results in clones hybridizing with varying intensity and the ones showing the strongest signals are picked. Genes that are most strongly expressed in the original population comprise the majority of the total mRNA pool and thus give a strong signal in this selection.

The inserts in clones with the strongest signals are sequenced from the 3'end of the insert using any standard DNA sequencing technique as known in the art. This provides a first identification of each clone and allows the exclusion of identical clones. The frequency with which each desired clone is represented in the cDNA lambda-bank is determined by hybridizing the bank against a clone-specific PCR probe. The desired clones are those which, in addition to having the strongest signals as above, are also represented at the highest frequencies in the cDNA bank, since this implies that the abundancy of the mRNA in the population was relatively high and thus that the promoter for that gene was highly active under the growth conditions. Thus, the relevance of this approach and any clone identified therefrom can be double-checked: the intensity of the hybridization signal of a specific clone should correlate positively with the frequency with which that clone is found in the cDNA bank. The inserts of the clones selected in this manner, such inserts corresponding to the cDNA sequences, may be used as probes to isolate the corresponding genes and their promoters from a chromosomal bank, such as one cloned into lambda as above.

The method of the invention is not limited to *Trichoderma*, but would be using for cloning genes from any host, or from a specific tissue with such host, from which a cDNA bank may be constructed, including, prokaryote (bacterial) hosts, and any eukaryotic host plants, mammals, insects, yeast, and any cultured cell populations.

For example, using the method of the invention, five genes that express relatively high levels of mRNA in *Trichoderma reesei* when such *Trichoderma*

are grown on glucose were identified. These genes were sequenced and identified as clone cDNA33, cDNA1, cDNA10, cDNA12, and cDNA15. When used to screen a *Trichoderma* chromosomal lambda-bank, the corresponding genes and their promoters were identified. Such genes and promoters (or portions thereof) may then be subcloned into any desired vector, such as the pSP73 vector (Promega, Madison, WI, USA).

According to the invention, the clones containing the genes and their promoters (or parts of them) highly expressed in *Trichoderma* grown on glucose are represented as follows:

Plasmid	Figure	cDNA	Figure	SEQ ID No
pTHN1	1A	cDNA33	1B	1
pEA33	2	cDNA33	1B	1
pTHN3	3A	cDNA1	3B	2
pEA10	4A	cDNA10	4B	3
pEA12	5A	cDNA12	5B	4
pEA155	6A	cDNA15	6B	5

One of the genes isolated according to the invention as being highly expressed when *Trichoderma* was grown on glucose has been identified as the one encoding *Trichoderma* translation elongation factor 1α (*tef1*). In addition, four other, new genes have been identified for the first time that are highly expressed on glucose in *Trichoderma*.

These data show that the method used in this invention resulted in isolating five genes, one of which (*tef1*) is known to be efficiently expressed in other organisms. However, the *tef1* gene was not the most highly expressed of the five genes isolated from the *Trichoderma* cDNA bank by the method of the invention.

Of the five genes isolated, only *tef1* shows a relevant degree of homology to any known protein sequences. All of the genes isolated are also expressed on other carbon sources and would not have been found with the

classical method of differential cloning. This shows the importance of the method used in this invention in isolation of the most suitable genes for a specific purpose, such as for isolation of strong promoters for expression on glucose containing medium.

The promoter of any of these genes may be operably linked to a sequence heterologous to such promoter, and especially heterologous to the host *Trichoderma*, for expression of such gene from a *Trichoderma* host that is grown on glucose. Preferably, the coding sequence provides a secretion signal for secretion of the recombinant protein into the medium.

Use of the promoters of the invention allow for the expression of genes from *Trichoderma* under conditions in which there are no cellulases and relatively few proteases. Thus, for the first time, recombinant genes can be highly expressed on *Trichoderma* using a glucose-based growth medium.

The promoters of the invention, while being strongly expressed on glucose (that is, when the filamentous fungal host is grown on medium providing glucose as a carbon and energy source), are not repressed in the absence of glucose. In addition, they are active when the *Trichoderma* host is grown on carbon sources other than glucose.

The glucose promoters of the invention, and those identified by the methods of the invention, can be used to produce enzymes native to *Trichoderma* itself, especially of those capable of hydrolysing different kinds of plant material. On glucose, the fungus does not naturally produce these enzymes and consequently one or more specific hydrolytic enzymes could be produced on glucose medium free from other plant material hydrolyzing enzymes. This would result in an enzyme preparate or enzyme mixtures for specific applications.

II. Modification of the Cellobiohydrolase I Promoter

This invention also describes a method for the modification of the *cellobiohydrolase 1* promoter (*cbh1*) such that the activity of the promoter is retained but the promoter no longer is repressed when cells are grown on glucose-containing medium. Essentially, the DNA motif that imparted glucose repression has been identified and removed from this promoter, allowing production of desired proteins whose coding sequences are operably linked to the promoter in suitable hosts, such as *Trichoderma*. Such a modified *cbh1* promoter is termed a derepressed *cbh1* promoter. As above, when the recombinant organisms obtained from transformation with such constructs are cultivated on glucose containing medium, any protein, including a cellulase may be produced without production of other plant material hydrolysing enzymes, especially of native cellulases.

Isolated glucose promoters or derepressed *cbh1* promoter can be used for instance to produce separate individual cellulases in hosts grown on glucose without any simultaneous production of other hydrolases such as other cellulases, hemicellulases, xylanases etc. or to produce heterologous proteins in varying growth media.

III. Preparation of Coding Sequences Operably Linked to the Promoter Sequences of the Invention

The process for genetically engineering a coding sequence, for expression under a promoter of the invention, is facilitated through the isolation and partial sequencing of pure protein encoding an enzyme of interest or by the cloning of genetic sequences which are capable of encoding such protein with polymerase chain reaction technologies; and through the expression of such genetic sequences. As used herein, the term "genetic sequences" is intended to refer to a nucleic acid molecule (preferably DNA). Genetic sequences that are capable of encoding a protein are derived from a

variety of sources. These sources include genomic DNA, cDNA, synthetic DNA, and combinations thereof. The preferred source of genomic DNA is a fungal genomic bank. The preferred source of the cDNA is a cDNA bank prepared from fungal mRNA grown in conditions known to induce expression of the desired gene to produce mRNA or protein. However, since the genetic code is universal, a coding sequence from any host, including prokaryotic (bacterial) hosts, and any eukaryotic host plants, mammals, insects, yeasts, and any cultured cell populations would be expected to function (encode the desired protein).

Genomic DNA may or may not include naturally occurring introns. Moreover, such genomic DNA may be obtained in association with the 5′ promoter region of the gene sequences and/or with the 3′ transcriptional termination region. According to the invention however, the native promoter region would be replaced with a promoter of the invention.

Such genomic DNA may also be obtained in association with the genetic sequences which encode the 5′ non-translated region of the mRNA and/or with the genetic sequences which encode the 3′ non-translated region. To the extent that a host cell can recognize the transcriptional and/or translational regulatory signals associated with the expression of the mRNA and protein, then the 5′ and/or 3′ non-transcribed regions of the native gene, and/or, the 5′ and/or 3′ non-translated regions of the mRNA may be retained and employed for transcriptional and translational regulation.

Genomic DNA can be extracted and purified from any host cell, especially a fungal host cell, which naturally expresses the desired protein by means well known in the art. A genomic DNA sequence may be shortened by means known in the art to isolate a desired gene from a chromosomal region that otherwise would contain more information than necessary for the utilization of this gene in the hosts of the invention. For example, restriction digestion may be utilized to cleave the full-length sequence at a desired location. Alternatively, or in addition, nucleases that cleave from the 3′-end of a DNA molecule may be used to digest a certain sequence to a shortened

form, the desired length then being identified and purified by gel electrophoresis and DNA sequencing. Such nucleases include, for example, Exonuclease III and *Bal*31. Other nucleases are well known in the art.

For cloning into a vector, such suitable DNA preparations (either genomic DNA or cDNA) are randomly sheared or enzymatically cleaved, respectively, and ligated into appropriate vectors to form a recombinant gene (either genomic or cDNA) bank.

A DNA sequence encoding a desired protein or its functional derivatives may be inserted into a DNA vector in accordance with conventional techniques, including blunt-ending or staggered-ending termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed by Maniatis, T., (Maniatis, T. *et al., Molecular Cloning (A Laboratory Manual)*, Cold Spring Harbor Laboratory, second edition, 1988) and are well known in the art.

Libraries containing sequences coding for the desired gene may be screened and the desired gene sequence identified by any means which specifically selects for a sequence coding for such gene or protein such as, for example, a) by hybridization with an appropriate nucleic acid probe(s) containing a sequence specific for the DNA of this protein, or b) by hybridization-selected translational analysis in which native mRNA which hybridizes to the clone in question is translated *in vitro* and the translation products are further characterized, or, c) if the cloned genetic sequences are themselves capable of expressing mRNA, by immunoprecipitation of a translated protein product produced by the host containing the clone.

Oligonucleotide probes specific for a certain protein which can be used to identify clones to this protein can be designed from the knowledge of the amino acid sequence of the protein or from the knowledge of the nucleic acid sequence of the DNA encoding such protein or a related protein. Alternatively, antibodies may be raised against purified forms of the protein

and used to identify the presence of unique protein determinants in transformants that express the desired cloned protein. When an amino acid sequence is listed horizontally, unless otherwise stated, the amino terminus is intended to be on the left end and the carboxy terminus is intended to be at the right end. Similarly, unless otherwise stated or apparent from the context, a nucleic acid sequence is presented with the 5' end on the left.

Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid. Peptide fragments may be analyzed to identify sequences of amino acids that may be encoded by oligonucleotides having the lowest degree of degeneracy. This is preferably accomplished by identifying sequences that contain amino acids which are encoded by only a single codon.

Although occasionally an amino acid sequence may be encoded by only a single oligonucleotide sequence, frequently the amino acid sequence may be encoded by any of a set of similar oligonucleotides. Importantly, whereas all of the members of this set contain oligonucleotide sequences which are capable of encoding the same peptide fragment and, thus, potentially contain the same oligonucleotide sequence as the gene which encodes the peptide fragment, only one member of the set contains the nucleotide sequence that is identical to the exon coding sequence of the gene. Because this member is present within the set, and is capable of hybridizing to DNA even in the presence of the other members of the set, it is possible to employ the unfractionated set of oligonucleotides in the same manner in which one would employ a single oligonucleotide to clone the gene that encodes the peptide.

Using the genetic code, one or more different oligonucleotides can be identified from the amino acid sequence, each of which would be capable of encoding the desired protein. The probability that a particular oligonucleotide will, in fact, constitute the actual protein encoding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic cells. Using "codon usage rules," a single oligonucleotide

sequence, or a set of oligonucleotide sequences, that contain a theoretical "most probable" nucleotide sequence capable of encoding the protein sequences is identified.

The suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a fragment of a certain gene (or which is complementary to such an oligonucleotide, or set of oligonucleotides) may be synthesized by means well known in the art (see, for example, *Oligonucleotides and Analogues, A Practical Approach*, F. Eckstein, ed., 1992, IRL Press, New York) and employed as a probe to identify and isolate a clone to such gene by techniques known in the art. Techniques of nucleic acid hybridization and clone identification are disclosed by Maniatis, T., *et al.*, in: *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (1982)), and by Hames, B.D., *et al.*, in: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985)). Those members of the above-described gene bank which are found to be capable of such hybridization are then analyzed to determine the extent and nature of coding sequences which they contain.

To facilitate the detection of a desired DNA coding sequence, the above-described DNA probe is labeled with a detectable group. Such detectable group can be any material having a detectable physical or chemical property. Such materials have been well-developed in the field of nucleic acid hybridization and in general most any label useful in such methods can be applied to the present invention. Particularly useful are radioactive labels, such as ^{32}P, ^{3}H, ^{14}C, ^{35}S, ^{125}I, or the like. Any radioactive label may be employed which provides for an adequate signal and has a sufficient half-life. If single stranded, the oligonucleotide may be radioactively labelled using kinase reactions. Alternatively, polynucleotides are also useful as nucleic acid hybridization probes when labeled with a non-radioactive marker such as biotin, an enzyme or a fluorescent group.

Thus, in summary, the elucidation of a partial protein sequence, permits the identification of a theoretical "most probable" DNA sequence, or

a set of such sequences, capable of encoding such a peptide. By constructing an oligonucleotide complementary to this theoretical sequence (or by constructing a set of oligonucleotides complementary to the set of "most probable" oligonucleotides), one obtains a DNA molecule (or set of DNA molecules), capable of functioning as a probe(s) for the identification and isolation of clones containing a gene.

In an alternative way of cloning a gene, a bank is prepared using an expression vector, by cloning DNA or, more preferably cDNA prepared from a cell capable of expressing the protein into an expression vector. The bank is then screened for members which express the desired protein, for example, by screening the bank with antibodies to the protein.

The above discussed methods are, therefore, capable of identifying genetic sequences that are capable of encoding a protein or biologically active or antigenic fragments of this protein. The desired coding sequence may be further characterized by demonstrating its ability to encode a protein having the ability to bind antibody in a specific manner, the ability to elicit the production of antibody which are capable of binding to the native, non-recombinant protein, the ability to provide a enzymatic activity to a cell that is a property of the protein, and the ability to provide a non-enzymatic (but specific) function to a recipient cell, among others.

In order to produce the recombinant protein in the vectors of the invention, it is desirable to operably link such coding sequences to the glucose regulatable promoters of the invention. When the coding sequence and the operably linked promoter of the invention are introduced into a recipient eukaryotic cell (preferably a fungal host cell) as a non-replicating DNA (or RNA), non-integrating molecule, the expression of the encoded protein may occur through the transient (nonstable) expression of the introduced sequence.

Preferably the coding sequence is introduced on a DNA molecule, such as a closed circular or linear molecule that is incapable of autonomous replication, Preferably, a linear molecule that integrates into the host chromosome. Genetically stable transformants may be constructed with vector systems, or

transformation systems, whereby a desired DNA is integrated into the host chromosome. Such integration may occur *de novo* within the cell or, be assisted by transformation with a vector which functionally inserts itself into the host chromosome.

The gene encoding the desired protein operably linked to the promoter of the invention may be placed with a transformation marker gene in one plasmid construction and introduced into the host cells by transformation, or, the marker gene may be on a separate construct for co-transformation with the coding sequence construct into the host cell. The nature of the vector will depend on the host organism. In the practical realization of the invention the filamentous fungus *Trichoderma* has been employed as a model. Thus, for *Trichoderma* and especially for *T. reesei*, vectors incorporating DNA that provides for integration of the expression cassette (the coding sequence operably linked to its transcriptional and translational regulatory elements) into the host's chromosome are preferred. It is not necessary to target the chromosomal insertion to a specific site. However, targeting the integration to a specific locus may be achieved by providing specific coding or flanking sequences on the recombinant construct, in an amount sufficient to direct integration to this locus at a relevant frequency.

Cells that have stably integrated the introduced DNA into their chromosomes are selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector in the chromosome, for example the marker may provide biocide resistance, e.g., resistance to antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transformation. A genetic marker especially for the transformation of the hosts of the invention is *amdS*, encoding acetamidase and thus enabling *Trichoderma* to grow on acetamide as the only nitrogen source. Selectable markers for use in transforming filamentous fungi include, for example, acetamidase (the *amdS* gene), benomyl resistance, oligomycin resistance,

hygromycin resistance, aminoglycoside resistance, bleomycin resistance; and, with auxotrophic mutants, ornithine carbamoyltransferase (OCTase or the *argB* gene). The use of such markers is also reviewed in Finkelstein, D.B. in: *Biotechnology of Filamentous Fungi: Technology and Products,* Chapter 6, Finkelstein, D.B. *et al.*, eds., Butterworth-Heinemann, publishers, Stoneham, MA, (1992), pp. 113-156).

To express a desired protein and/or its active derivatives, transcriptional and translational signals recognizable by an appropriate host are necessary. The cloned coding sequences, obtained through the methods described above, and preferably in a double-stranded form, may be operably linked to sequences controlling transcriptional expression in an expression vector, and introduced into a host cell, either prokaryote or eukaryote, to produce recombinant protein or a functional derivative thereof. Depending upon which strand of the coding sequence is operably linked to the sequences controlling transcriptional expression, it is also possible to express antisense RNA or a functional derivative thereof.

Expression of the protein in different hosts may result in different post-translational modifications which may alter the properties of the protein. Preferably, the present invention encompasses the expression of the protein or a functional derivative thereof, in eukaryotic cells, and especially in fungus.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains expression control sequences which contain transcriptional regulatory information and such sequences are "operably linked" to the nucleotide sequence which encodes the polypeptide.

An operable linkage is a linkage in which a sequence is connected to a regulatory sequence (or sequences) in such a way as to place expression of the sequence under the influence or control of the regulatory sequence. Two DNA sequences (such as a coding sequence and a promoter region sequence linked to the 5' end of the coding sequence) are said to be operably linked if induction of promoter function results in the transcription of mRNA encoding the desired protein and if the nature of the linkage between the two DNA

sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the expression regulatory sequences to direct the expression of the protein, antisense RNA, or (3) interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably linked to a DNA sequence if the promoter was capable of effecting transcription of that DNA sequence.

The precise nature of the regulatory regions needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5′ non-transcribing and 5′ non-translating (non-coding) sequences involved with initiation of transcription and translation respectively, such as the TATA box, capping sequence, CAAT sequence, and the like, with those elements necessary for the promoter sequence being provided by the promoters of the invention. Such transcriptional control sequences may also include enhancer sequences or upstream activator sequences, as desired.

Expression of a protein in eukaryotic hosts such as fungus requires the use of regulatory regions functional in such hosts, and preferably fungal regulatory systems. A wide variety of transcriptional and translational regulatory sequences can be employed, depending upon the nature of the host. Preferably, these regulatory signals are associated in their native state with a particular gene which is capable of a high level of expression in the host cell.

In eukaryotes, where transcription is not linked to translation, such control regions may or may not provide an initiator methionine (AUG) codon, depending on whether the cloned sequence contains such a methionine. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis in the host cell. Promoters from filamentous fungal genes which encode a mRNA product capable of translation are preferred, and especially, strong promoters can be employed provided they also function as promoters in the host cell.

As is widely known, translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence

which encodes the desired protein, or a functional derivative thereof, does not contain any intervening codons which are capable of encoding a methionine. The presence of such codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as the protein-coding DNA sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the protein-coding sequence).

It may be desired to construct a fusion product that contains a partial coding sequence (usually at the amino terminal end) of a protein and a second coding sequence (partial or complete) of a second protein. The first coding sequence may or may not function as a signal sequence for secretion of the protein from the host cell. For example, the sequence coding for desired protein may be linked to a signal sequence which will allow secretion of the protein from, or the compartmentalization of the protein in, a particular host. Such fusion protein sequences may be designed with or without specific protease sites such that a desired peptide sequence is amenable to subsequent removal. In a preferred embodiment, the native signal sequence of a fungal protein is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the peptide that is operably linked to it. *Aspergillus* leader/secretion signal elements also function in *Trichoderma*.

If desired, the non-transcribed and/or non-translated regions 3′ to the sequence coding for a desired protein can be obtained by the above-described cloning methods. The 3′-non-transcribed region may be retained for its transcriptional termination regulatory sequence elements, or for those elements which direct polyadenylation in eukaryotic cells. Where the native expression control sequences signals do not function satisfactorily in a host cell, then sequences functional in the host cell may be substituted.

The vectors of the invention may further comprise other operably linked regulatory elements such as DNA elements which confer antibiotic resistance, or origins of replication for maintenance of the vector in one or more host cells.

Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Once the vector or DNA sequence containing the construct(s) is prepared for expression, the DNA construct(s) is introduced into an appropriate host cell by any of a variety of suitable means, including transformation. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. If this medium includes glucose, expression of the cloned gene sequence(s) results in the production of the desired protein, or in the production of a fragment of this protein as desired. This expression can take place in a continuous manner in the transformed cells, or in a controlled manner, for example, by induction of expression.

Fungal transformation is carried out also accordingly to techniques known in the art, for example, using, for example, homologous recombination to stably insert a gene into the fungal host and/or to destroy the ability of the host cell to express a certain protein.

Fungi useful as recombinant hosts for the purpose of the invention include, e.g., *Trichoderma*, *Aspergillus*, *Claviceps purpurea*, *Penicillium chrysogenum*, *Magnaporthe grisea*, *Neurospora*, *Mycosphaerella spp.*, *Collectotrichum trifolii*, the dimorphic fungus *Histoplasmia capsulatum*, *Nectia haematococca* (anamorph:*Fisarium solani* f. sp. *phaseoli* and f. sp. *pisi*), *Ustilago violacea*, *Ustilago maydis*, *Cephalosporium acremonium*, *Schizophyllum commune*, *Podospora anserina*, *Sordaria macrospora*, *Mucor circinelloides*, and *Collectotrichum capsici*. Transformation and selection techniques for each of these fungi have been described (reviewed in Finkelstein, D.B. in: *Biotechnology of Filamentous Fungi: Technology and Products*, Chapter 6, Finkelstein, D.B. *et al.*, eds., Butterworth-Heinemann,

publishers, Stoneham, MA, (1992), pp. 113-156). Especially preferred are *Trichoderma reesei*, *T. harzianum*, *T. longibrachiatum*, *T. viride*, *T. koningii*, *Aspergillus nidulans*, *A. niger*, *A. terreus*, *A. ficum*, *A. oryzae*, *A. awamori* and *Neurospora crassa*.

The hosts of the invention are meant to include all *Trichoderma*. *Trichoderma* are classified on the basis of morphological evidence of similarity. *T. reesei* was formerly known as *T. viride* Pers. or *T. koningii* Oudem; sometimes it was classified as a distinct species of the *T. longibrachiatum* group. The entire genus *Trichoderma*, in general, is characterized by rapidly growing colonies bearing tufted or pustulate, repeatedly branched conidiophores with lageniform phialides and hyaline or green conidia borne in slimy heads (Bissett, J., Can. J. Bot. 62:924-931 (1984)).

The fungus called *T. reesei* is clearly defined as a genetic family originating from the strain QM6a, that is, a family of strains possessing a common genetic background originating from a single nucleus of the particular isolate QM6a. Only those strains are called *T. reesei*.

Classification by morphological means is problematic and the first recently published molecular data from DNA-fingerprint analysis and the hybridization pattern of the cellobiohydrolase 2 (*cbh2*) gene in *T. reesei* and *T. longibrachiatum* clearly indicates a differentiation of these strains (Meyer, W. *et al.*, *Curr. Genet.* *21*:27-30 (1992); Morawetz, R. *et al.*, *Curr. Genet.* *21*:31-36 (1992).

However, there is evidence of similarity between different *Trichoderma* species at the molecular level that is found in the conservation of nucleic acid and amino acid sequences of macromolecular entities shared by the various *Trichoderma* species. For example, Cheng, C., *et al.*, *Nucl. Acids. Res.* *18*:5559 (1990), discloses the nucleotide sequence of *T. viride cbh1*. The gene was isolated using a probe based on the *T. reesei* sequence. The authors note that there is a 95% homology between the amino acid sequences of the *T. viride* and *T. reesei* gene. Goldman, G.H. *et al.*, *Nucl. Acids Res.* *18*:6717

(1990), discloses the nucleotide sequence of phosphoglycerate kinases from *T. viride* and notes that the deduced amino acid sequence is 81% homologous with the phosphoglycerate kinase gene from *T. reesei*. Thus, the species classified to *T. viride* and *T. reesei* must genetically be very close to each other.

In addition, there is a high similarity of transformation conditions among the *Trichoderma*. Although practically all the industrially important species of *Trichoderma* can be found in the formerly discussed *Trichoderma* section *Longbrachiatum*, there are some other species of *Trichoderma* that are not assigned to this section. Such a species is, for example, *Trichoderma harzianum*, which acts as a biocontrol agent against plant pathogens. A transformation system has also been developed for this *Trichoderma* species (Herrera-Estrella, A. *et al.*, *Molec. Microbiol. 4*:839-843 (1990) that is essentially the same as that taught in the application. Thus, even though *Trichoderma harzianum* is not assigned to the section *Longibrachiatum*, the method used by Herrera-Estrella in the preparation of spheroplasts before transformation is the same. The teachings of Herrera-Estrella show that there is not a significant diversity of *Trichoderma* spp. such that the transformation system of the invention would not be expected to function in all *Trichoderma*.

Further, there is a common functionality of fungal transcriptional control signals among fungal species. At least three *A. nidulans* promoter sequences, *amdS*, *argB*, and *gpd*, have been shown to give rise to gene expression in *T. reesei*. For *amdS* and *argB*, only one or two copies of the gene are sufficient to being about a selectable phenotypes (Penttilä *et al.*, *Gene 61*:155-164 (1987). Gruber, F. *et al.*, *Curr. Genetic 18*:71-76 (1990) also notes that fungal genes can often be successfully expressed across different species. Therefore, it is to be expected that the glucose regulated promoters identified herein would be also regulatable by glucose in other fungi. Except for *cbh1*, it is understood that the glucose regulated promoters of the invention may not be directly regulated by glucose, but rather that they function regardless of its presence.

Many species of fungi, and especially *Trichoderma*, are available from a wide variety of resource centers that contain fungal culture collections. In addition, *Trichoderma* species are catalogued in various databases. These resources and databases are summarized by O'Donnell, K. *et al.*, in *Biochemistry of Filamentous Fungi: Technology and Products*, D.B. Fingelstein *et al.*, eds., Butterworth-Heinemann, Stoneham, MA, USA, 1992, pp. 3-39.

After the introduction of the vector and selection of the transformant, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene sequence(s) results in the synthesis and secretion of the desired heterologous or homologous protein, or in the production of a fragment of this protein, into the medium of the host cell.

In a preferred embodiment, the coding sequence is the sequence of an enzyme that is capable of hydrolysing lignocellulose. Examples of such sequences include a DNA sequence encoding cellobiohydrolase I (CBHI), cellobiohydrolase II (CBHII), endoglucanase I (EGI), endoglucanase II (EGII), endoglucanase III (EGIII), β-glucosidases, xylanases (including endoxylanases and β-xylosidase), side-group cleaving activities, (for example, α-arabinosidase, α-D-glucuronidase, and acetyl esterase), mannanases, pectinases (for example, endo-polygalacturonase, exo-polygalacturonase, pectinesterase, or, pectin and pectin acid lyase), and enzymes of lignin polymer degradation, (for example, lignin peroxidase LIII from *Phlebia radiata* (Saloheimo *et al.*, *Gene 85*:343-351 (1989)), or the gene for another ligninase, laccase or Mn peroxidase (Kirk, In: *Biochemistry and Genetics of Cellulose Degradation*, Aubert *et al.* (eds.), FEMS Symposium No. 43, Academic Press, Harcourt, Brace Jovanovitch Publishers, London. pp. 315-332 (1988))). The cloning of the cellulolytic enzyme genes has been described and recently reviewed (Teeri, T.T. in: *Biotechnology of Filamentous Fungi: Technology and Products*, Chapter 14, Finkelstein, D.B. *et al.*, eds., Butterworth-Heinemann, publishers, Stoneham, MA, (1992), pp. 417-445). The gene for the native

cellobiohydrolase CBHI sequence has been cloned by Shoemaker *et al.* (Shoemaker, S., *et al.*, *Bio/Technology 1*:691-696 (1983)) and Teeri *et al.* (Teeri, T., *et al.*, *Bio/Technology 1*:696-699 (1983)) and the entire nucleotide sequence of the gene is known (Shoemaker, S., *et al.*, *Bio/Technology 1*:691-696 (1983)). From *T. reesei*, the gene for the major endoglucanase (EGI) has also been cloned and characterized (Penttilä, M., *et al.*, *Gene 45*:253-263 (1986); Patent Application EP 137,280; Van Arstel, J.N.V., *et al.*, *Bio/Technology 5*:60-64). Other isolated cellulase genes include *cbh2* (Patent Application WO 85/04672; Chen, C.M., *et al.*, *Bio/Technology 5*:274-278 (1987)) and *egl3* (Saloheimo, M., *et al.*, *Gene 63*:11-21 (1988)). The genes for the two endo-β-xylanases of *T. reesei* (*xln1* and *xln2* have been cloned and described in applicants' copending application, U.S. 07/889,893, filed May 29, 1992. The xylanase proteins have been purified and characterized (Tenkanen, M. *et al.*, *Proceeding of the Xylans and Xylanases Symposium*, Wageningen, Holland (1991)).

The expressed protein may be isolated and purified from the medium of the host in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis, or the like. For example, the cells may be collected by centrifugation, or with suitable buffers, lysed, and the protein isolated by column chromatography, for example, on DEAE-cellulose, phosphocellulose, polyribocytidylic acid-agarose, hydroxyapatite or by electrophoresis or immunoprecipitation.

The manner and method of carrying out the present invention may be more fully understood by those of skill by reference to the following examples, which examples are not intended in any manner to limit the scope of the present invention or of the claims directed thereto.

Example 1

Isolation of Trichoderma reesei Genes Strongly Expressed on Glucose

For the isolation of glucose induced mRNA *Trichoderma reesei* strain QM9414 (Mandels, M. *et al.*, *Appl. Microbiol. 21*:152-154 (1971)) was grown in a 10 liter fermenter in glucose medium (glucose 60 g/l, Bacto-Peptone 5 g/l, Yeast extract 1 g/l, KH_2PO_4 4 g/l, $(NH_4)_2SO_4$ 4 g/l, $MgSO_4$ 0.5 g/l, $CaCl_2$ 0.5 g/l and trace elements $FeSO_4 \cdot 7H_2O$ 5 mg/l, $MnSO_4 \cdot H_2O$ 1.6 mg/l, $ZnSO_4 \cdot 7H_2O$ 1.4 mg/l, and $CoCl_2 \cdot 6H_2O$ 3.7 mg/l, pH 5.0-4.0). Glucose feeding (465g/20h) was started after 30 hours of growth. Mycelium was harvested at 45 hours of growth and RNA was isolated according to Chirgwin, J.M. *et al.*, *Biochem. J. 18*:5294-5299 (1979)). Poly A+ RNA was isolated from the total RNA by oligo(dT)-cellulose chromatography (Maniatis, T. *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982)) and cDNA synthesis and cloning of the cDNAs was carried out according to manufacturer's instructions into lambda-ZAP vector (ZAP-cDNA synthesis kit, Stratagene). The cDNA bank was transferred onto nitrocellulose filters and screened with ^{32}P-labelled single-stranded cDNA synthesized (Teeri, T.T. *et al.*, *Anal. Biochem. 164*:60-67 (1987)) from the same poly A+ RNA from which the bank was constructed. The labelled cDNA was relabelled with ^{32}P-dCTP (Random Primed DNA Labeling kit, Boehringer-Mannheim). The hybridization conditions were as described in Maniatis, T. *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). Fifty clones giving the strongest positive reaction were isolated and the cDNAs were subcloned *in vivo* into Bluescript SK(-) plasmid according to manufacturer's instructions (ZAP-cDNA synthesis kit, Stratagene).

To identify the clones and exclude the same ones they were all sequenced from the 3' end by using standard methods. The frequency of each specific clone in the cDNA lambda-bank was determined by hybridizing the bank with a clone specific PCR probe. The clones cDNA33, cDNA1,

cDNA10, cDNA12, cDNA15, showing the five highest frequencies corresponded to 1-3% of the total mRNA pool.

Example 2

Characterization of Isolated Glucose Expressed Trichoderma Genes and Their Promoters

The cDNAs of the clones cDNA33, cDNA1, cDNA10, cDNA12, and cDNA15 were used as probes to isolate the corresponding genes and promoters from a *Trichoderma* chromosomal lambda-bank prepared earlier (Vanhanen, S. *et al.*, *Curr. Genet. 15*:181-186 (1989)). On the basis of Southern analysis of restriction enzyme digestions carried out for the chromosomal lambda clones, the promoters and either the 5′ parts of the chromosomal genes or the whole genes were subcloned into pSP73 vector (Promega, Madison, USA) using appropriate restriction enzymes yielding the plasmids pTHN1 (Figure 1), pEA33 (Figure 2), pTHN3 (Figure 3), pEA10 (Figure 4), pEA12 (Figure 5) and pEA155 (Figure 6), corresponding to the clones cDNA33, cDNA1, cDNA10, cDNA12 and cDNA15, respectively. Sequences were obtained from the 5′ ends of the genes and from the promoters using primers designed from previously obtained sequences. The sequences of the isolated promoters and genes or parts of them (either obtained from cDNA or chromosomal DNA) are shown in SEQ ID1 for cDNA33, SEQ ID2 for cDNA1, SEQ ID3 for cDNA10, SEQ ID4 for cDNA12, and SEQ ID5 for cDNA15. Based on sequence similarity to known sequences in a protein data bank the clone cDNA33 could be identified as a translation elongation factor, TEF1α.

Example 3

Construction of Vectors for Expression of EGI-core under the tef1-Promoter in Trichoderma

A *Xho*I + *Dra*III fragment that is internal to the *egl1* cDNA [SEQ ID 16 and Figure 7A] sequence of plasmid pPLE3 (Figure 7) carrying the *Eco*RI-*Bam*HI fragment of *egl1* cDNA from pTTc11 (Penttilä *et al.*, *Gene 45*:253-263 (1986); Penttilä *et al.*, *Yeast 3*:175-185 (1987) inbetween the *cbh1* promoter and c. 700 nt long *Ava*II terminator fragment was replaced by a *Xho*I-*Dra*III fragment of cDNA from plasmid pEG131 (Nitisinprasert, S., *Reports from Department of Microbiology*, University of Helsinki (1990)). The pPEG131 insert sequence is *egl1* cDNA in which a STOP codon is constructed just before the hinge region of the *egl1* gene. The *cbh1* terminator sequence is Figure 7B [SEQ ID 23]. SEQ ID 23 is a shortened *cbh1* terminator sequence, similar to SEQ ID 24 (the "long" *cbh1* terminator but lacking 30 nucleotides at the 5′ end).

pPLE3 contains a pUC18 backbone, and carries the *cbh1* promoter inserted at the *Eco*RI site. The *cbh1* promoter is operably linked to the full length *egl1* cDNA coding sequence and to the *cbh1* transcriptional terminator. The ori and amp genes are from the bacterial plasmid.

The resulting plasmid pEM-3 (Figure 8) now carries a copy of *egl1* cDNA with a translational stop codon after the *egl1* core region (EGI amino acids 1-22 are the EGI signal sequence; EGI amino acids 23-393, terminating at a Thr, are considered the 'core' sequence). pEM-3 was then digested with *Eco*RI and *Sph*I and the released Bluescribe M13+ moiety (Vector Cloning Systems, San Diego, USA) of the plasmid was replaced by *Eco*RI and *Sph*I digested pAMD (Figure 8) containing a 3.4 kb *amdS* fragment from plasmid p3SR2 (Hynes, M.J. *et al.*, *Mol. Cell. Biol. 3*:1430-1439 (1983); Tilburn, J. *et al.*, *Gene 26*:205-221 (1983). This resulting plasmid pEM-3A (Figure 8) was digested with *Eco*RI and partially with *Ksp*I to release the 2.3 kb fragment carrying the *cbh1*-promotor and the 8.6 kb fragment carrying the rest of the

plasmid was purified from agarose gel. Based on the sequence data of the *tef1* promoter (SEQ ID1 bases 1-1234), two primers were designed (SEQ ID6 and SEQ ID7) and used in a PCR reaction to isolate a 1.2 kb promoter fragment adjacent to the translational start site of the *tef1* gene. The 5′ primer was

ACCGGAATTCATATCTAGAGGAGCCCGCGAGTTTGGATACGCC (SEQ ID6)

and the 3′ primer was

ACCGCCGCGGTTTGACGGTTTGTGTGATGTAGCG (SEQ ID7).

The bold and underlined GAATTC in the 5′ primer is an *Eco*RI site. The bold and underlined TCTAGA in the 5′ primer is an *Xba*I site. The bold and underlined CCGCGG in the 3′ primer is a *Sac*II site. This fragment was digested with *Eco*RI and partially with *Ksp*I and purified from agarose gel and ligated to the 8.6 kb pEM-3A fragment resulting in plasmid pTHN100B (Figure 9). This expression vector carries DNA encoding the EGI-core construction operably linked to the *tef1* promoter; this plasmid also carries an *amdS* marker gene for selection of *Trichoderma* transformants.

Example 4

Transformation of Trichoderma, Purification of the EGI-Core Producing Clones and Their Analysis

Trichoderma reesei strain QM9414 was transformed essentially as described (Penttilä, M. *et al.*, *Gene 61*:155-164 (1987) using 6-10 μg of the plasmid pTHN100B. The Amd$^+$ transformants obtained were streaked twice onto slants containing acetamide (Penttilä, M. *et al. Gene 61*:155-164 (1987)). Thereafter spore suspensions were made from transformants grown on Potato Dextrose agar (Difco). EGI-core production was tested by slot blotting with EGI specific antibody from 50 ml shake flask cultures carried out in minimal medium (Penttilä, M. *et al. Gene 61*:155-164 (1987)) supplemented with 5% glucose and using additional glucose feeding (total amount of fed glucose was 6 ml of 20% glucose). The spore suspensions of the EGI-core producing clones were purified to single spore cultures on Potato Dextrose agar plates.

EGI-core production was analyzed again from these purified clones as described above (Figure 10).

Example 5

Characterization of EGI-core produced by Trichoderma Grown on Glucose

EGI-core producing strain pTHN100B-16c was grown in a 10 liter fermenter in glucose medium as described earlier in Example 1 except that yeast extract was left out and glucose feeding was 555g/22h. The culture supernatant was separated from the mycelium by centrifugation. The secretion of EGI-core by *Trichoderma* was verified by Western blotting by conventional methods running concentrated culture supernatants on SDS-PAGE and treating the blotted filter with monoclonal EGI-core specific antibodies (Figure 11 and Figure 12). The enzyme activity was shown semiquantitatively in a microtiter plate assay by using the concentrated culture supernatants and 3 mM chloronitrophenyl lactocide as a substrate and measuring the absorbance at 405 nm (Clayessens, M. *et al., Biochem. J. 261*:819-825 (1989).

Example 6

Construction of β-Galactosidase Expression Vectors with Truncated Fragments of the cbh1-Promoter

The vector pMLO16 (Figure 13) contains a 2.3 kb *cbh1* promoter fragment ([SEQ ID18, Figure 13A) starting at 5′ end from the *Eco*RI site, isolated from chromosomal gene bank of *Trichoderma reesei* (Teeri, T. *et al., J. Bio/Technology 1*:696-699 (1983)), a 3.1 kb *Bam*HI fragment of the *lacZ* gene from plasmid pAN924-21 (van Gorcom *et al., Gene 40*:99-106 (1985)) and a 1.6 kb *cbh1* terminator (Figure 13B, [SEQ ID 24]) starting from 84 bp upstream from the translation stop codon and extending to a *Bam*HI site at the 3′ end (Shoemaker, S. *et al., Bio/Technology 1*:691-696 (1983); Teeri, T. *et al., Bio/Technology 1*:696-699 (1983)). These pieces were linked to a 2.3

kb long *Eco*RI-*Pvu*II region of pBR322 (Sutcliffe, J.G., *Cold Spring Harbor Symp. Quant. Biol. 43*:77-90 (1979)) generating junctions as shown in Figure 13. The exact in frame joint between the 2.3 kb *cbh1* promoter and the 3.1 kb *lacZ* gene was constructed by using an oligo depicted in Figure 13. A polylinker shown in Figure 13 was cloned into the single internal *Xba*I site in the *cbh1* promoter for the purpose of promoter deletions. A short *Sal*I linker shown in Figure 13 was cloned into the joint between the pBR322 and *cbh1* promoter fragments so that the expression cassette can be released from the vector by restriction digestion with *Sal*I and *Sph*I. Progressive unidirectional deletions were introduced to the *cbh1* promoter by cutting the vector with *Kpn*I and *Xho*I and using the Erase-A-Base System (Promega, Madison, USA) according to manufacturer's instructions. Plasmids obtained from different deletion time points were transformed into the *E. coli* strain DH5α (BRL) by the method described in (Hanahan D., *J. Mol. Biol. 166*:557-580 (1983)) and the deletion end points were sequenced by using standard methods.

Example 7

Transformation of Trichoderma, Isolation of the β-Galactosidase Producing Clones and Their Analysis

Trichoderma reesei strain QM9414 was transformed with expression vectors for β-galactosidase containing either the intact 2.3 kb *cbh1* promoter or truncated versions of it, generated as explained in Example 6. Twenty μg of the plasmids were digested with *Sal*I and *Sph*I to release the expression cassettes from the vectors and these mixtures were cotransformed to *Trichoderma* together with 3 μg of plasmid p3SR2 (Hynes, M.J. *et al.*, *Mol. Cell. Biol. 3*:1430-1439 (1983)) containing the acetamidase gene. The transformation method was that described in (Penttilä, M. *et al. Gene 61*:155-164 (1987)) and the Amd^+ transformants were screened as described earlier in Example 4. The β-galactosidase production of the Amd^+ transformants was tested by inoculating spore suspensions on microtiter plate wells containing

solid minimal medium (Penttilä, M. *et al. Gene 61*:155-164 (1987)) supplemented with 2% glucose, 2% fructose and 0.2% peptone and pH adjusted to 7. After 24 h incubation in 28°C, 10 µl of the chromogenic substrate X-gal (20 mg/ml) was added to each well and the formation of blue color was followed as an indication of β-galactosidase activity. An intense blue color could be detected in transformants transformed with a plasmid pMLO16del5(11) (Figure 14) containing a 1110 bp deletion in the *cbh1* promoter beginning from the promoter internal polylinker and ending 385 bp before the translation initiation site (Figure 15). The sequence of this truncated promoter is provided as SEQ ID19 (Figure 15A).

Example 8

Production of CBHI on Glucose with the Glucose-Derepressed cbh1-Promoter

For the production of CBHI on glucose an expression plasmid pMLO 17 (Figure 16) was constructed. The plasmid pMLO16del5(11) was digested with the enzymes *Ksp*I (the first nucleotide of the recognition sequence is at the position -16 from the ATG) and *Xma*I (the first nucleotide of the recognition sequence is 76 nucleotides downstream from the translation stop codon of the *cbh1* gene). The vector part containing the shortened *cbh1* promoter, the *cbh1* terminator and the pBR322 sequence was ligated to the chromosomal *cbh1* gene isolated as a *Ksp*I-*Xma*I-fragment from the chromosomal gene bank of *Trichoderma reesei* (Teeri, T. *et al.*, *Bio/Technology 1*:696-699 (1983)). The sequence of this fragment is provided as the underlined portion of Figure 16A ([SEQ ID17]). The plasmid pMLO17 was transformed to the *Trichoderma reesei* strain QM 9414 and the Amd$^+$ transformants were screened as described earlier in example 7. CBHI production was tested from 40 transformants in microtiter plate cultures (200 µl; 3 days) carried out in minimal medium (Penttilä, M. *et al. Gene 61*:155-164 (1987) supplemented with 3% glucose and using additional glucose

feeding (total amount of fed glucose was 6 mg/200 µl culture). The culture supernatants were slot blotted on nitrocellulose filters and CBHI was detected with specific antibody. The spore suspensions of the 10 best CBHI producing transformants were purified to single spore cultures on plates containing acetamide and Triton X-100 (Penttilä, M. *et al.*, *Gene 61*:155-164 (1987)). Thirty single spore cultures were tested for CBHI production in shake flask cultivations (50 ml; 6 days) carried out in the same medium as described above. The total amount of fed glucose was 1.8g/50ml culture. Dilutions of the culture supernatants were slot blotted and CBHI was detected with specific antibody (Figure 17).

Example 9

β-Galactosidase Expression Vectors with Specific Mutations in cbh1 Promoter to Release Glucose Repression

Three 6 bp sequences found in *cbh1* promoter similar to binding sites of *Saccharomyces cerevisiae* glucose repressor protein MIG1 (Nehlin & Ronne, *EMBO J.* 9:2891-2899 (1990); Nehlin *et al.*, *EMBO J. 10*:3373-3377 (1991)) were changed into other nucleotides to study the functionality of these mig-like sequences in mediating the glucose repression of the native *cbh1* promoter of *Trichoderma reesei*. To construct β-galactosidase expression vectors with *cbh1* promoters carrying specific mutations, sequence alterations were made into primers (specifically: TCT TCA AGA ATT GCT CGA CCA ATT CTC ACG GTG AAT GTA GG (SEQ ID 8); ACA CAT CTA GAG GTG ACC TAG GCA TTC TGG CCA CTA GAT ATA TAT TTA GAA GGT TCT TGT AGC TCA AAA GAG C (SEQ ID 9); GGG AAT TCT CTA GAA ACG CGT TGG CAA ATT ACG GTA CG (SEQ ID 10); GGG AAT TCG GTC ACC TCT AAA TGT GTA ATT TGC CTG CTT GAC C (SEQ ID 11); GGG AAT TCG GTC ACC TCT AAA TGT GTA ATT TGC CTG CTT GAC CGA TCT AAA CTG TTC GAA GCC CGA ATG TAG G (SEQ ID 12); GGG AAT TCT TCT AGA TTG CAG AAG CAC GGC AAA GCC CAC TTA CCC (SEQ ID 13); TAG CGA ATT CTA GGT CAC CTC TAA AGG TAC CCT GCA GCT CGA GCT AG (SEQ ID 14); and GGG AAT TCA TGA TGC GCA GTC CGC GG (SEQ ID 15);

these primers were specific for the *cbh1* promoter and the *cbh1* promoter internal polylinker and were used in PCR amplification of *cbh1* promoter sequences for cloning.

pMLO16 (Figure 13) was used as a PCR template with the appropriate primers to yield a 770 bp fragment A (primers TAG CGA ATT CTA GGT CAC CTC TAA AGG TAC CCT GCA GCT CGA GCT AG (SEQ ID 14) and GGG AAT TCT CTA GAA ACG CGT TGG CAA ATT ACG GTA CG (SEQ ID 10), beginning at the polylinker at -1500 and ending at -720 upstream of ATG, and a 720 bp fragment B (primers GGG AAT TCT TCT AGA TTG CAG AAG CAC GGC AAA GCC CAC TTA CCC (SEQ ID 13) and GGG AAT TCA TGA TGC GCA GTC CGC GG (SEQ ID 15)), beginning at -720 and ending at *Ksp*I at -16. Fragments A and B were purified from agarose gel and digested with *Bst*EII-*Xba*I and *Xba*I-*Ksp*I respectively, ligated to the 7.8 kb fragment of pMLO16 to produce pMI-24. The resulting *cbh1* promoter carries a sequence alteration (genomic sequence 5′ GTGGGG, altered sequence: 5′ TCTAGA) at position -720 to -715 upstream of the translation initiation codon of intact *cbh1* promoter (Figure 18). The sequence of the altered *cbh1* promoter in pMI-24 is provided in Figure 18A and SEQ ID20.

pMLO16del0(2) (Figure 19) containing a 460 bp deletion in the *cbh1* promoter beginning from the promoter internal polylinker and ending 1025 bp before the translation initiation site was constructed as described in Example 6 and used as a PCR template with primers (TCT TCA AGA ATT GCT CGA CCA ATT CTC ACG GTG AAT GTA GG (SEQ ID 8) and ACA CAT CTA GAG GTG ACC TAG GCA TTC TGG CCA CTA GAT ATA TAT TTA GAA GGT TCT TGT AGC TCA AAA GAG C (SEQ ID 9)) to yield a 800 bp fragment C, beginning from the 5′ end of *cbh1* promoter and ending at the promoter internal polylinker. Fragment C was purified from agarose gel, digested with *Sal*I-*Xba*I and ligated to the 7.6 kb *Sal*I-*Xba*I fragment of pMLO16del0(2) to produce pMI-25. The *cbh1* promoter of pMI-25 has a sequence alteration (genomic sequence: 5′GTGGGG, altered sequence: 5′TCTAAA) at position -1505-1500 upstream of the translation initiation codon of intact *cbh1* promoter (Figure 18).

pMLO16del0(2) was used as a PCR template to yield a 750 bp fragment D (primers GGG AAT TCG GTC ACC TCT AAA TGT GTA ATT TGC CTG CTT GAC CGA TCT AAA CTG TTC GAA GCC CGA ATG TAG G (SEQ ID 12) and GGG AAT TCA TGA TGC GCA GTC CGC GG (SEQ ID 15)), beginning from the promoter internal polylinker and ending at *Ksp*I at -16. Fragment D was purified from agarose gel, digested with *Bst*EII-*Ksp*I and ligated to the 7.8 kb *Bst*EII-*Ksp*I fragment of pMI-25 to produce pMI-26. The *cbh1* promoter of pMI-26 has sequence alterations at positions -1505-1500 (genomic sequence: 5'GTGGGG, altered sequence: 5'TCTAAA) and -1001-996 (genomic sequence: 5'CTGGGG, altered sequence: 5'TCTAAA) upstream of the translation initiation codon of intact *cbh1* promoter (Figure 18).

pMLO16del0(2) was used as a PCR template to yield a 280 bp fragment E (primers GGG AAT TCT CTA GAA ACG CGT TGG CAA ATT ACG GTA CG (SEQ ID 10) and GGG AAT TCG GTC ACC TCT AAA TGT GTA ATT TGC CTG CTT GAC C (SEQ ID 11)), beginning from the promoter internal polylinker and ending at -720 and a 720 bp fragment F (primers GGG AAT TCT TCT AGA TTG CAG AAG CAC GGC AAA GCC CAC TTA CCC (SEQ ID 13) and GGG AAT TCA TGA TGC GCA GTC CGC GG (SEQ ID 15)), beginning at -720 and ending at *Ksp*I at -16. Fragments D and E were purified from agarose gel, digested with *Bst*EII-*Xba*I and *Xba*I-*Ksp*I respectively and ligated to the 7.8 kb BstEII-*Ksp*I fragment of pMI-25 to produce pMI-27. The *cbh1* promoter of pMI-27 has sequence alterations at positions -1505-1500 (genomic sequence: 5'GTGGGG, altered sequence: 5'TCTAAA) and -720-715 (genomic sequence: 5'GTGGGG, altered sequence: 5'TCTAGA) upstream of the translation initiation codon of intact *cbh1* promoter (Figure 18). The sequence of the altered *cbh1* promoter of pMI-27 is shown in Figure 18C and SEQ ID21.

pMLO16del0(2) was used as a PCR template to yield a 280 bp fragment G (primers GGG AAT TCT CTA GAA ACG CGT TGG CAA ATT ACG GTA CG (SEQ ID 10) and GGG AAT TCG GTC ACC TCT AAA TGT GTA ATT TGC CTG CTT GAC CGA TCT AAA CTG TTC GAA GCC CGA ATG TAG G (SEQ ID 12)), beginning from the promoter internal polylinker and ending at -720 and a 720

bp fragment H (primers GGG AAT TCT TCT AGA TTG CAG AAG CAC GGC AAA GCC CAC TTA CCC (SEQ ID 13) and GGG AAT TCA TGA TGC GCA GTC CGC GG (SEQ ID 15)), beginning at -720 and ending at KspI at -16. Fragments G and H were purified from agarose gel, digested with BstEII-XbaI and XbaI-KspI respectively and ligated to the 7.8 kb BstEII-KspI fragment of pMI-25 to produce pMI-28. The *cbh1* promoter of pMI-28 has sequence alterations at positions -1505-1500 (genomic sequence: 5'GTGGGG, altered sequence: 5'TCTAAA), -1001-996 (genomic sequence: 5'CTGGGG, altered sequence: 5'TCTAAA), and -720-715 (genomic sequence: 5'GTGGGG, altered sequence: 5'TCTAGA) upstream of the translation initiation codon of intact *cbh1* promoter (Figure 18). The sequence of the altered *cbh1* promoter of pMI-28 is shown in Figure 18C and SEQ ID22.

All PCR amplified DNA fragments and ligation joints were sequenced using standard methods to ensure that the mutations were present and no other nucleotides were changed. Transformation of *Trichoderma reesei* QM9414 with the vectors mentioned above, isolation of β-galactosidase producing clones and their analysis was done as described in Example 7. After addition of X-gal, an intense blue color was detected on glucose grown transformant colonies as an indication of β-galactosidase activity in transformants transformed with the plasmids pMI-24, pMI-27 and pMI-28 (Figure 20), indicating that altering the *cbh1* promoter according to any of those mutations was sufficient to allow for expression of proteins in *Trichoderma* under the *cbh1* promoter in the presence of glucose.

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3461 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGCCGTGACG ACAGAAACGG AGCCCGCGAG TTTGGATACG CCGCTGAAAT GGGGCTTGAC 60

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CGACCAAACG CAACCATCAA ATCAATCAGA TGGCAATGAC GCACCACCGC CCCAGCAGCG 180

CGAACCGCCG ACGAAGAAGC CATGGACGCG CTCCTCGGCA AGACGCCCAA GGAACAGAAA 240

AAAGTAATCT CCGCACCCGT ATCAGAAGAC GACGCCTACC GCCGCGACGT CGAAGCCTCC 300

GGCGCGGTGT CCACGCTCCA GGATTACGAA GACATGCCCG TCGAGGAGTT TGGCGCCGCC 360

CTCCTCCNNN GCATGGGCTG GAACGGGGAA GCCCGCGGCC CGCCGGTCAA GCAGGTCAAG 420

AGGCGGCAGA ACAGGCTCGG CCTCGGCGCC AAGGAGCTCA AGGAGGAAGA GGACCTCGGC 480

GGGTGGAACC AGAACGGCAA GAAAAAGTCG AGGCCSCGCG GCTGAGCGAG TATCGGAGGG 540

AGGAGAGCAA GCGCAAGGAA GGCCGGGGGC ATGAGGACAG CTATAAACGA GAGAGGGAGC 600

GCGAACGGAT CGCGAGAGGG ATCACTACAG GGAGCGAGAC CGGGACAGGG ATCGCGATTA 660

TAGGGATCGG GATAGGGATA GACATCGGGA CCACGATAGG CACAGGGACC GACATCGCGA 720

CTCTGACCGG CACCATCGAC GATGAAGGAG CTTTTGCATT CTTCTCTTCG TCAACCACTT 780

TTGAGACTAA CATTAACCAT GCCGTTTTCT TGAAAAGCTT GTACTCATCA TGATGTTTTT 840

AAGCAAATAG GCGACAGGCG TACAGACACC TTAATATCAC ATAGAGGCAC GGCACACATA 900

CGTCTTGGAG AAGACACGTA CTTACGAATG ATGGGAGAAT TACCTACTCT GACTTGTGTA 960

AATTAGAATA TCAATGACAC TATGTATATT CAGTCGAGCT GCGAATGGTC ACACATTGTC 1020

TGATCTGCGA ATTTGTATGT GCTGCCTCTC CCTCTGACCT TCTGGTCTGG TGATACCATC 1080

CTCCCTCAGT TTGGATCATC GCCTTATTCT TCTTCCCTCT TCTGCATCTG CTTCCTGCTC 1140

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AGCGATGCTA ACCATATTCC CTCGAACAGG AAGCCGCCGA ACTCGGCAAG GGTTCCTTCA 1920

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GGTCGTTACC TTCGCTCCCT CCAACGTCAC CACTGAAGTC AAGTCCGTCG AGATGCACCA 2700

CGAGCAGCTC GCTGAGGGCC AGCCTGGTGA CAACGTTGGT TTCAACGTGA AGAACGTTTC 2760

CGTCAAGGAA ATCCGCCGTG GCAACGTTGC CGGTGACTCC AAGAACGACC CCCCCATGGG 2820

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CGAGAAGATC GACCGCCGTA CCGGTAAGGC TACCGAGTCT GCCCCCAAGT TCATCAAGTC 3000

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CGACTACCCT CCCCTGGGTC GTTTCGCCGT CCGTGACATG CGCCAGACCG TCGCTGTCGG 3120

TGTCATCAAG GCCGTCGAGA AGTCCTCTGC CGCCGCCGCN AAGGTCACCA AGTCCGCTGC 3180

CAAGGCCGCC AAGAAATAAG CGATACCCAT CATCAACACC TGATGTTCTG GGGTCCCTCG 3240

TGAGGTTTCT CCAGGTGGGC ACCACCATGC GCTCACTTCT ACGACGAAAC GATCAATGTT 3300

GCTATGCATG AGSACTCGAC TATGAATCGA GGCACGGTTA ATTGAGAGGC TGGGAATAAG 3360

GGTTCCATCA GAACTTCTCT GGGAATGCAA AACAAAAGGG AACAAAAAAA CTAGATAGAA 3420

GTGAATTCAT GACTTCGACA ACCAAAAAAA AAAAAAAAAA A 3461

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1636 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGTCTGAAGG ACGTGGAATG ATGGACTTAA TGACAAGAGT TGCCTGGCTA TTGAGCTCTG 60

GTACATGGAT CTCGAACTGA GAGCGTACAA GTTACATGTA GTAAATCTAG TAGATCTCGC 120

TGAAAGCCCT CTTTCCCGGT AGAAACACCA CCAGCGTCCC GTAGGACAAG ATCCTGTCGA 180

TCTGAGCACA TGAATTGCTT CCCTGGATCT GGCGCTGCAT CTGTTTCCCC AGACAATGAT 240

GGTAGCAGCG CATGGAAGAA CCCGGTTGTT CGGAATGTCC TTGTGCTAAC AGTGGCATGA 300

TTTTACGTTG CGGCTCATCT CGCCTTGGCA CCGGACCTCA GCAAATCTTG TCACAACAGC 360

AATCTCAAAC AGCCTCATGG TTCCCAGATT CCCTGATTCA GAACTCTAGA GCGGCAGATG 420

TCAAACGATT CTGACCTAGT ACCTTGAGCA TCCCTTTCGG ATCCGGCCCA TGTTCTGCCT 480

GCCCTTCTGA GCACAGCAAA CAGCCCAAAA GGCGCCGGCC GATTCCTTTC CCGGGATGCT 540

CCGGAGTGGC ACCACCTCCC AAAACAAGCA ACCTTGAACC CCCCCCCCAA ATCAACTGAA 600

GCGCTCTTCG CCTAACCAGC ATAAGCCCCC CCCAGGATCG TTAGGCCAAG TGGTAGGGCC 660

AGCCAATTAG CGAGNGGCCA TTTGGAGGTC ATGGGCGCAG AATGTCCTGA CAGTGGTATG 720

ATATTGACTG CCCGGTGTGT GTGGCATCTG GCCATAATCG CAGGCTGAGG CGAGGAAGTC 780

TCGTGAGGAT GTCCCGACTT TGACATCATG AGGGAGTGAG AAACTGAAGA GAAGGAAAGC 840

TTCGAAGGTT CGATAAGGGA TGATTTGCAT GGCGGGCGAC AGGATGCGAT GGCTCGTTGG 900

GATACATAAT GCTTGGGTTG GAAGCGATTC CAGGTCGTCT TTTTTTGGTT CATCATCACA 960

GCATCAACAA GCAACGATAC AAGCAATCCA CTGAGGATTA CCTCTCAACT CAACCACTTT 1020

CCAAACCATC TCAACTCCCT AAGATTCTTT CAGTGTATTA TCACTAGGAT TTTTCCCAAG 1080

CCGGCTTCAA AACACACAGA TAAACCACCA ACTCTACAAC CAAAGACTTT TTGATCAATC 1140

CAACAACTTC TCTCAACATG TCTGCTGCAA CCGTCACCCG CACTGCAACC GCCGCTGTTC 1200

GCAGACCCGG CTTCTTCATG CAAGTCCGAC GGATGGGACG CTCATTCGAG CACCAGCCCT 1260

TTGAGCGACT CTCCGCCACC ATGAAGCCTG CACGACCCGA CTATGCTAAG CAAGTCGTCT 1320

GGACGGCTGG CAAGTTTGTC ACTTATGTTC CTCTTTTCGG CGCCATGCTT ACCTGGCCTG 1380

CGCTCGCCAA STGGGCTCTG GACGGACACA TCGGACGGTG GTAAAAGATC AGACTCTTGT 1440

CGAGGCAACG GGGAATAGAC AGGACAGCAA AAAAGATATC TCCGGATAGA AGTGTCCATC 1500

TTTCGACTTG TATATATATA TATGCTATAC TCTGGGGGCG TTTGGATGGA CTTTGGGCAC 1560

GAAGCATACT TTGGCGCAAC GCAGATACTT TAATCTGATT CCTTTTGTTA ATTCAAAAAA 1620

AAAAAAAAAA AAAAAA 1636

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2868 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTTGTATGGC TGGATCTCGA AAGGCCCTTG TCATCGCCAA GCGTGGCTAA TATCGAATGA 60

GGGACACCCA CTTGCATATC TCCTGATCAT TCAAACGACA AGTGTGAGGT AGGCAATCCT 120

CGTATCCCAT TGCTGGGCTG AAAGCTTCAC ACGTATCGCA TAAGCGTCTC CAACCAGTGC 180

TTAGGTGACC CTTAAGGATA CTTACAGTAA GACTGTATTA AGTCAGTCAC TCTTTCACTC 240

GGGCTTTGAA TACGATCCTC AATACTCCCG ATAACAGTAA GAGGATGATA CAGCCTGCAG 300

TTGGCAAATG TAAGCGTAAT TAAACTCAGC TGAACGGCCC TTGTTGAAAG TCTCTCTCGA 360

TCAAAGCAAA GCTATCCACA GACAAGGGTT AAGCAGGCTC ACTCTTCCTA CGCCTTGGAT 420

ATGCAGCTTG GCCAGCATCG CGCATGGCCA ATGATGCACC CTTCACGGCC CAACGGATCT 480

CCCGTTAAAC TCCCCTGTAA CTTGGCATCA CTCATCTGTG ATCCCAACAG ACTGAGTTGG 540

GGGCTGCGGC TGGCGGATGT CGGAGCAAAG GATCACTTCA AGAGCCCAGA TCCGGTTGGT 600

CCATTGCCAA TGGATCTAGA TTCGGCACCT TGATCTCGAT CACTGAGACA TGGTGAGTTG 660

CCCGGACGCA CCACAACTCC CCCTGTGTCA TTGAGTCCCC ATATGCGTCT TCTCAGCGTG 720

CAACTCTGAG ACGGATTAGT CCTCACGATG AAATTAACTT CCAGCTTAAG TTCGTAGCCT 780

TGAATGAGTG AAGAAATTTC AAAAACAAAC TGAGTAGAGG TCTTGAGCAG CTGGGGTGGT 840

ACGCCCCTCC TCGACTCTTG GGACATCGTA CGGCAGAGAA TCAACGGATT CACACCTTTG 900

GGTCGAGATG AGCTGATCTC GACAGATACG TGCTTCACCA CAGCTGCAGC TACCTTTGCC 960

CAACCATTGC GTTCCAGGAT CTTGATCTAC ATCACCGCAG CACCCGAGCC AGGACGGAGA 1020

GAACAATCCG GCCACAGAGC AGCACCGCCT TCCAACTCTG CTCCTGGCAA CGTCACACAA 1080

CCTGATATTA GATATCCACC TGGGTGATTG CCATTGCAGA GAGGTGGCAG TTGGTGATAC 1140

CGACTGGCCA TGCAAGACGC GGCCGGGCTA GCTGAAATGT CCCCGAGAGG ACAATTGGGA 1200

GCGTCTATGA CGGCGTGGAG ACGACGGGAA AGGACTCAGC CGTCATGTTG TGTTGCCAAT 1260

TTGAGATTGT TGACCGGGAA AGGGGGGACG AAGAGGATGG CTGGGTGAGG TGGTATTGGG 1320

AGGATGCATC ATTCGACTCA GTGAGCGATG TAGAGCTCCA AGAATATAAA TATCCCTTCT 1380

CTGTCTTCTC AAAATCTCCT TCCATCTTGT CCTTCATCAG CACCAGAGCC AGCCTGAACA 1440

CCTCCAGTCA ACTTCCCTTA CCAGTACATC TGAATCAACA TCCATTCTTT GAAATCTCAC 1500

CACAACCACC ATCTTCTTCA AAATGAAGTT CTTCGCCATC GCCGCTCTCT TTGCCGCCGC 1560

TGCCGTTGCC CAGCCTCTCG AGGACCGCAG CAACGGCAAC GGCAATGTTT GCCCTCCCGG 1620

CCTCTTCAGC AACCCCCAGT GCTGTGCCAC CCAAGTCCTT GGCCTCATCG GCCTTGACTG 1680

CAAAGTCCGT AAGTTGAGCC ATAACATAAG AATCCTCTTG ACGGAAATAT GCCTTCTCAC 1740

TCCTTTACCC CTGAACAGCC TCCCAGAACG TTTACGACGG CACCGACTTC CGCAACGTCT 1800

GCGCCAAAAC CGGCGCCCAG CCTCTCTGCT GCGTGGCCCC CGTTGTAAGT TGATGCCCCA 1860

GCTCAAGCTC CAGTCTTTGG CAAACCCATT CTGACACCCA GACTGCAGGC CGGCCAGGCT 1920

CTTCTGTGCC AGACCGCCGT CGGTGCTTGA GATGCCCGCC CGGGGTCAAG GTGTGCCCGT 1980

GAGAAAGCCC ACAAAGTGTT GATGAGGACC ATTTCCGGTA CTGGGAAAGT TGGCTCCACG 2040

TGTTTGGGCA GGTTTGGGCA AGTTGTGTAG ATATTCCATT CGTACGCCAT TCTTATTCTC 2100

CAATATTTCA GTACACTTTT CTTCATAAAT CAAAAAGACT GCTATTCTCT TTGTGACATG 2160

CCGGAAGGGA ACAATTGCTC TTGGTCTCTG TTATTTGCAA GTAGGAGTGG GAGATTCGCC 2220

TTAGAGAAAG TAGAGAAGCT GTGCTTGACC GTGGTGTGAC TCGACGAGGA TGGACTGAGA 2280

GTGTTAGGAT TAGGTCGAAC GTTGAAGTGT ATACAGGATC GTCTGGCAAC CCACGGATCC 2340

TATGACTTGA TGCAATGGTG AAGATGAATG ACAGTGTAAG AGGAAAAGGA AATGTCCGCC 2400

TTCAGCTGAT ATCCACGCCA ATGATACAGC GATATACCTC CAATATCTGT GGGAACGAGA 2460

CATGACATAT TTGTGGGAAC AACTTCAAAC AGCGAGCCAA GACCTCAATA TGCACATCCA 2520

AAGCCAAACA TTGGCAAGAC GAGAGACAGT CACATTGTCG TCGAAAGATG GCATCGTACC 2580

CAAATCATCA GCTCTCATTA TCGCCTAAAC CACAGATTGT TTGCCGTCCC CCAACTCCAA 2640

AACGTTACTA CAAAAGACAT GGGCGAATGC AAAGACCTGA AAGCAAACCC TTTTTGCGAC 2700

TCAATTCCCT CCTTTGTCCT CGGAATGATG ATCCTTCACC AAGTAAAAGA AAAAGAAGAT 2760

TGAGATAATA CATGAAAAGC ACAACGGAAA CGAAAGAACC AGGAAAAGAA TAAATCTATC 2820

ACGCACCTTG TCCCCACACT AAAAGCAACA GGGGGGGTAA AATGAAAT 2868

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2175 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAAAAGCTAG AACGAGACGA TTCCGGCCCG GCAAACCAGG CCGAGTGACG GGAGCATTTC 60

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CATGATTTCA CTCGGCAAAC TCTGGCTACA ATTTTCAGGC GGCGAGTTCC GATACAAGGG      120
AAATCTATTA CCCACAGACG AACGGGAATC GGTGATGAGT GGTTTCTTGT AAGTCAACAT      180
TGAGCTAGAT AATTCCGGGC GAGATCAAGA TGCCATACTT TGATTGATGA AAAATCAATG      240
TCAGGCGTAA GTCTCTTCAA GCTCGCCCAG TCCTCTGTAT GTAACAGCAA TCGCAATTCC      300
GAAATGTGCC GAGCCAATGG AACATGCGTG TCTTTCTCTT TTCACACACA TCCAGTTCGA      360
GAGTCTTCTC TTCATCGTTT CATCGAATCC CTTCCCCTCC AGCTATTCAC CCAGCCGAGC      420
CCTTCAGCGC ACCAGCGTAT GTATGTACCC TCGGCTAAGA CGCAACAGAA GCATCATCAA      480
TATACCTGAT GTACTACTAT CTACTATGAA GCCCAAAAAC CCCTTCGCAG CCCAAATGTA      540
ACCCAAGCAA CGAATCCCCA ATAAGAGACA ATCCTCAGTG ACCCCCAGAA GAGCACAGAA      600
TCGAGCTGGT CCTGGTGGGT CGCATTGAGA CCGGTGGAGA TGCGTTCGAT TCGACTGCCG      660
GAGCTCCCGG GAAGCCGGCA GATGGTCCCA TGCGATGCCC TGCACCGTTT TTGTGAATCG      720
TCGGCATCGC GAGAAGTGGC CTGCTATGAC GTCGCTTGCA GCTTGGCCGC TCTGTTCGAA      780
GTTTTTCGAT GTTTTTCTTC ATGCGGGAGA AAGAAAACAT CAGATGACAT GATTATCCGA      840
ATGGATGGCG GGAGTTATCG TGGTGACGGC TGCTTCATGA GATGAGTATA AATGAGCTTG      900
TTCGCTCAGC GTGTCATGGA TCTTGTCCAG CTCCAAAGCA TCGGCTTCAG CATCCATCCG      960
CTTGAACAGA CAGGCACCAG CTTGAATCAG AAGCATACCC TTGATTTGAT ACTCTCTTGG     1020
GAAAAAACAC CACCATCTGT GTAATACTTT GATACCCCCA AAGCTCAAAC GACCGCTTGT     1080
ACATACAATA ACACCGCCAC AATGTTCGCC AACTTGACGC ACGCTACCCT GCGATTCATC     1140
GCCTTCTTCA ACCACCTGAT GATCCTGGCC TCATCAGCCA TCGTCACCGG CCTCGTATCC     1200
TGGTTCCTCG ACAAGTACGA CTACCGCGGC GTGAACATTG TCTACCAGGA AGTCATCGTA     1260
TGTCCTCCCA AGCACCACAT CAAACACACC CCATACCTTG GCTCTCCTCA GCTCCGTCGA     1320
AGCACATAAT ACTAACGCAT GCAACAACTA GGCCACCATA ACTCTGGGCT TCTGGCTCGT     1380
TGGTGCCGTC TTGCCCCTCG TTGGCAGATA CCGCGGCCAC CTGGCCCCTC TCAACCTCAT     1440
CTTCTCCTAC CTCTGGCTCA CCTCTTTCAT CTTCTCCGCG CAGGACTGGA GCAGCGACAA     1500
GTGCAGCTTC GGCCAGCCTG GCGAGGGCCA CTGCAGCCGC AAGAAGGCCA TTGAATCCTT     1560
CAACTTTATC GCATTGTAAG TGCCTACAAG TAATTTGCTA TGTATATGGG AGAGAGAGAG     1620
AAGAAGAAGA ATATGGCTCT AACATGGCAT CTCTACAGCT TCTTCCTCCT CTGCAACACC     1680
CTGGTTGAGA TGCTCCTGCT CCGCGCCGAG TATGCTACCC CCGTTGCTGC TGCTCACAAC     1740
AAGGAGATTT CTGCCGGCCG CCCCTCTGAC AACTCTGTCT AAATAACAAT AGACATGCAT     1800
AGATGAACGG AGACCACTTC TACTTTCTTT GCGAGTTCCT GATCCGTTGA CCTGCAGGTC     1860
GACBBBBBCC GCGCTCGCAT GGTTCATCTG CTACAACAAC ACAATGACAA TCCGAACCAG     1920
TCAATAAACC TCGACAACAC GACGAGTACT TTTGCGGATA GAAAGATACC CATTACACAG     1980
GAGATCAAAT GGGGAAATTG GAAGTGTATG GATGGACGCC CGTGTATAAT GAGGTTGTGA     2040
ACGGGATGGG AGGCAATGAA TAATGGATAA TGAGGTAATG GATAGATTCG GTCGTTTTGA     2100
TACCACAGCT GCACTCTGCT CTACGTCTGT CATTAATGAT ACATACAAAT GATACCTTAT     2160
ACGCTAAAAA AAAAA                                                      2175
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2737 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCTAGAATCT CTTCGAGATG GCCGAGAAAG GCTTGTTTTT CTCTCCTTCT TCAAACTGGC 60
CACTGTTTGT TTTCAAACTT GGGGTTTCGT GGGGCTTTTG GGGGCATGTC TGCCAGGTCT 120
CCCGTAGGCT GGACAGCCAA AGCCTCACTA CAAACAGGCA GTTGTCAATA GATTGATGTC 180
TGAGATGGAT GGTTTTATGT TTGGGGGAGG TCATGTATGT ATTTATCTAT ATTTGCAAAG 240
ATGATCCATG AGTCAGACTT GCACAGGTTT CTCGTGCGCT GGATAAATCT TGTTGGAGTG 300
CGGGTGAGGT GGTGGATGGC ATTCAACCCA CAGCAACACT TGCCCAGGGG GATGTACTGC 360
AGCGATTTGT TTCCCTTCGA GTATTAGATG ATGATGCCGA ACAGACAAAT TTGAGCCTCG 420
CTGCTCTCGG ATGTCGGGTT TCTCTTGTGT GCCGGTGATG TGTGATGGCC TGGCCCGCAA 480
AGAGAGCGAA AAACATGCTC AAAATGTAGC ACACGGCGAC TTCTCGGACA CTTGCGTACC 540
TTGAGAGACA AGCAGACTAC AGGGATGACG AGTAATACGA CAGAGCGATA CGACACAGCT 600
ATACGACACA GCTAAGAAAA TAAAGGTATT AGTACTACTA ATTGATTACC TACTACCTAG 660
ATATATACTA TACCTTATAT TTTATATGTG TGTGTGTGTG TATGTATATG CCTTACCTTA 720
TGCTTCGCAA AGAAGAGAAA CTAAAACGCC TCCTGGCTAC CTACCTACCT CTACCTTGTA 780
AGAGATGGAA TAATGTGGCC GCGCGTAAAG TAGGTACTGG ATATACAGGT CCTGAACATG 840
GCCCTGAATC CTGCCAGGCA GCCACCTCAC CCCTTCCGCA GGTATTTATG TAGCCCACAG 900
CTCCTCCAGA GACGATGCCG AGATGCCTCA TGCAGTCTAC CTACAAAGCC AGCAGTTTCA 960
CGCTTGACTC TCACTCTTGA TTGAATTCCC TCCCTCCCAT AATACCAATT GGCGTTCAAC 1020
GATTGCCAGC AGAATGGCCG CCCAACACGA CGTCGAGGCC ATGGCAAAGT CCATGTCCGA 1080
CTTTTTCAAG GACACGGCCC AAAAGCAGGA CTCGACCAAG CATGACTTTG TCCAAGCCTC 1140
GCACGGCATC ATGAGGGCCA TTGTCGAGCC GCTCGTCACC CAGATGGGCT TCCGCGAGAC 1200
CCTCACCGAG CCCGTCGTCT TGCTCGACAG CGCGTGCGGA GCGGGCGTGC TGACGCAGGA 1260
GGTGCAGGCG GCGCTGCCAA AGGAGCTTCT GGAGAGGAGC TCGTTTACGT GTGCGGACAA 1320
TGCCGAGGGC TTGGTGGACG TGGTGAAGAG GAGGATTGAT GAGGAGAAGT GGGTGAATGC 1380
AGAGGCCAAG GTCCTTGATG CCCTGGTGAG TATATACATA TATATCTATA TCTATATAGA 1440
TATATATATG CCTTTGACTC CCCCCTTTAC ATGTCCTACG GCTGCTGATT GATTGATTGA 1500
TGTGGTGATG GTGATGTCCC AGAACACGGG GCTCCCAGAC AACTCCTTCA CCCATGTGGG 1560
CATTGCCCTG GCACTGCACA TCATCCCCGA TCCAGATGCC GTCGTCAAAG GTAAACAATC 1620
ACCAGCGTCA CTGCAAAGAG AGATTACGGG ATATCATATA CTGAAACCAA AGCCCAGACT 1680
GCATCAGAAT GCTCAAGCCA GGCGGCATCT TTGGCGCATC GACATGGCCC AAGGCCAGCG 1740
CCGACATGTT CTGGATCGCC GACATGCGCA CCGCCCTGCA GTCGCTCCCC TTTGACGCGC 1800

CGCTGCCAGA CCCGTTCCCC ATGCAGCTGC ACACCTCGGG CCACTGGGAC GACGCCGCCT 1860

GGGTCGAGAA GCATCTCGTC GAGGATCTGG GGCTGGCCAA CGTCTGTGTG AGGGAGCCGG 1920

CGGGCGAGTA CAGCTTTGCG AGCGCGGACG AGTTCATGGC GACGTTTCAG ATGATGCTGC 1980

CGTGGATTAT GAAGACGTTT TGGAGCGAGG AGGTGAGGGA GAAGCATTCG GTCGACGAGG 2040

TCAAGGAGTT GGTGAAGAGG CATCTGGAGG ACAAGTATGG GGGGAAGGGA TGGACCATTA 2100

AGTGGCGGGT GATTACCATG ACTGCGACTG CGAGCAAGTG AGGGAGGGCA TCTGCTCATG 2160

ATTATGTGAC AGCGAGCCAG TAGAGAGCCA TATTGTTGTC TTCAGAATGT GAGGACCGTG 2220

ATGGTTGGTG TTTGTTGGAG TGATAACTCG TGGGTGTTGC TATTTGCATG TGAGACGATG 2280

AACCATGCGC ACCAGCCACA ATCACTGTCC CCCACCTTAC CTACCAACTT CAAGTTACCA 2340

CCTTACCTTT ACCTGATCTA GCACTGTGGC GCAGCTTGGT TTGACTGCTA GGTACCTACC 2400

TAGTAGTAAT CAGGTACATT CTTCATCCCT GTGTCCTGGT GTCGCAGTTG CAGCTTGTCT 2460

TATCGCTGTG GCCACGCATC GAGTGGCAGC ATCTTCAACT TCAAGTCCCG TCGGTCGCAC 2520

TCTGGCCACG TCGCAGATGG ATCGCAGCGG GATCTGAACC GCTCGCTCGG CAACTGATAC 2580

CAAGTCAACA AACACACGAG ACGACGGGAC GCTGATATAA NNNNGAGGAG GGTAAGAGAA 2640

CTCTACGAGG GGCGGAAACT TGGTCCGACA ATTTCCCTCC CATCTTCACC CTCGACTCGA 2700

ACTCGAACTC GATAGCCGCA CCCTCGACCG ATTGCCC 2737

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ACCGGAATTC ATATCTAGAG GAGCCCGCGA GTTTGGATAC GCC 43

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACCGCCGCGG TTTGACGGTT TGTGTGATGT AGCG 34

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TCTTCAAGAA TTGCTCGACC AATTCTCACG GTGAATGTAG G 41

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACACATCTAG AGGTGACCTA GGCATTCTGG CCACTAGATA TATATTTAGA AGGTTCTTGT 60

AGCTCAAAAG AGC 73

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGAATTCTC TAGAAACGCG TTGGCAAATT ACGGTACG 38

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGGAATTCGG TCACCTCTAA ATGTGTAATT TGCCTGCTTG ACC 43

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGGAATTCGG TCACCTCTAA ATGTGTAATT TGCCTGCTTG ACCGATCTAA ACTGTTCGAA 60

GCCCGAATGT AGG 73

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGGAATTCTT CTAGATTGCA GAAGCACGGC AAAGCCCACT TACCC 45

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TAGCGAATTC TAGGTCACCT CTAAAGGTAC CCTGCAGCTC GAGCTAG 47

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGGAATTCAT GATGCGCAGT CCGCGG 26

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1588 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCCCCCTATC TTAGTCCTTC TTGTTGTCCC AAAATGGCGC CCTCAGTTAC ACTGCCGTTG 60

ACCACGGCCA TCCTGGCCAT TGCCCGGCTC GTCGCCGCCC AGCAACCGGG TACCAGCACC 120

CCCGAGGTCC ATCCCAAGTT GACAACCTAC AAGTGTACAA AGTCCGGGGG GTGCGTGGCC 180

CAGGACACCT CGGTGGTCCT TGACTGGAAC TACCGCTGGA TGCACGACGC AAACTACAAC 240

TCGTGCACCG TCAACGGCGG CGTCAACACC ACGCTCTGCC CTGACGAGGC GACCTGTGGC 300

AAGAACTGCT TCATCGAGGG CGTCGACTAC GCCGCCTCGG GCGTCACGAC CTCGGGCAGC 360

AGCCTCACCA TGAACCAGTA CATGCCCAGC AGCTCTGGCG GCTACAGCAG CGTCTCTCCT 420

CGGCTGTATC TCCTGGACTC TGACGGTGAG TACGTGATGC TGAAGCTCAA CGGCCAGGAG 480

CTGAGCTTCG ACGTCGACCT CTCTGCTCTG CCGTGTGGAG AGAACGGCTC GCTCTACCTG 540

TCTCAGATGG ACGAGAACGG GGGCGCCAAC CAGTATAACA CGGCCGGTGC CAACTACGGG 600

AGCGGCTACT GCGATGCTCA GTGCCCCGTC CAGACATGGA GGAACGGCAC CCTCAACACT 660

AGCCACCAGG GCTTCTGCTG CAACGAGATG GATATCCTGG AGGGCAACTC GAGGGCGAAT 720

GCCTTGACCC CTCACTCTTG CACGGCCACG GCCTGCGACT CTGCCGGTTG CGGCTTCAAC 780

CCCTATGGCA GCGGCTACAA AAGCTACTAC GGCCCCGGAG ATACCGTTGA CACCTCCAAG 840

ACCTTCACCA TCATCACCCA GTTCAACACG GACAACGGCT CGCCCTCGGG CAACCTTGTG 900

AGCATCACCC GCAAGTACCA GCAAAACGGC GTCGACATCC CCAGCGCCCA GCCCGGCGGC 960

GACACCATCT CGTCCTGCCC GTCCGCCTCA GCCTACGGCG GCCTCGCCAC CATGGGCAAG 1020

GCCCTGAGCA GCGGCATGGT GCTCGTGTTC AGCATTTGGA ACGACAACAG CCAGTACATG 1080

AACTGGCTCG ACAGCGGCAA CGCCGGCCCC TGCAGCAGCA CCGAGGGCAA CCCATCCAAC 1140

ATCCTGGCCA ACAACCCCAA CACGCACGTC GTCTTCTCCA ACATCCGCTG GGGAGACATT 1200

GGGTCTACTA CGAACTCGAC TGCGCCCCCG CCCCCGCCTG CGTCCAGCAC GACGTTTTCG 1260

ACTACACGGA GGAGCTCGAC GACTTCGAGC AGCCCGAGCT GCACGCAGAC TCACTGGGGG 1320

CAGTGCGGTG GCATTGGGTA CAGCGGGTGC AAGACGTGCA CGTCGGGCAC TACGTGCCAG 1380

TATAGCAACG ACTACTACTC GCAATGCCTT TAGAGCGTTG ACTTGCCTCT GGTCTGTCCA 1440

GACGGGGGCA CGATAGAATG CGGGCACGCA GGGAGCTCGT AGACATTGGG CTTAATATAT 1500

AAGACATGCT ATGTTGTATC TACATTAGCA AATGACAAAC AAATGAAAAA GAACTTATCA 1560

AGCAAAAAAA AAAAAAAAAA AAAAAAAA 1588

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1820 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCGCGGACTG CGCATCATGT 1740

ATCGGAAGTT GGCCGTCATC TCGGCCTTCT TGGCCACAGC TCGTGCTCAG TCGGCCTGCA 1800

CTCTCCAATC GGAGACTCAC CCGCCTCTGA CATGGCAGAA ATGCTCGTCT GGTGGCACTT 1860

GCACTCAACA GACAGGCTCC GTGGTCATCG ACGCCAACTG GCGCTGGACT CACGCTACGA 1920

ACAGCAGCAC GAACTGCTAC GATGGCAACA CTTGGAGCTC GACCCTATGT CCTGACAACG 1980

AGACCTGCGC GAAGAACTGC TGTCTGGACG GTGCCGCCTA CGCGTCCACG TACGGAGTTA 2040

CCACGAGCGG TAACAGCCTC TCCATTGGCT TTGTCACCCA GTCTGCGCAG AAGAACGTTG 2100

GCGCTCGCCT TTACCTTATG GGCAGCGACA CGACCTACCA GGAATTCACC CTGCTTGGCA 2160

ACGAGTTCTC TTTCGATGTT GATGTTTCGC AGCTGCCGTA AGTGACTTAC CATGAACCCC 2220

TGACGTATCT TCTTGTGGGC TCCCAGCTGA CTGGCCAATT TAAGGTGCGG CTTGAACGGA 2280

GCTCTCTACT TCGTGTCCAT GGACGCGGAT GGTGGCGTGA GCAAGTATCC CACCAACACC 2340

GCTGGCGCCA AGTACGGCAC GGGGTACTGT GACAGCCAGT GTCCCCGCGA TCTGAAGTTC 2400

ATCAATGGCC AGGCCAACGT TGAGGGCTGG GAGCCGTCAT CCAACAACGC AAACACGGGC 2460

ATTGGAGGAC ACGGAAGCTG CTGCTCTGAG ATGGATATCT GGGAGGCCAA CTCCATCTCC 2520

GAGGCTCTTA CCCCCCACCC TTGCACGACT GTCGGCCAGG AGATCTGCGA GGGTGATGGG 2580

TGCGGCGGAA CTTACTCCGA TAACAGATAT GGCGGCACTT GCGATCCCGA TGGCTGCGAC 2640

TGGAACCCAT ACCGCCTGGG CAACACCAGC TTCTACGGCC CTGGCTCAAG CTTTACCCTC 2700

GATACCACCA AGAAATTGAC CGTTGTCACC CAGTCCGAGA CGTCGGGTGC CATCAACCGA 2760

TACTATGTCC AGAATGGCGT CACTTTCCAG CAGCCCAACG CCGAGCTTGG TAGTTACTCT 2820

GGCAACGAGC TCAACGATGA TTACTGCACA GCTGAGGAGG CAGAATTCGG CGGATCCTCT 2880

TTCTCAGACA AGGGCGGCCT GACTCAGTTC AAGAAGGCTA CCTCTGGCGG CATGGTTCTG 2940

GTCATGAGTC TGTGGGATGA TGTGAGTTTG ATGGACAAAC ATGCGCGTTG ACAAAGAGTC 3000

AAGCAGCTGA CTGAGATGTT ACAGTACTAC GCCAACATGC TGTGGCTGGA CTCCACCTAC 3060

CCGACAAACG AGACCTCCTC CACACCCGGT GCCGTGCGCG GAAGCTGCTC CACCAGCTCC 3120

GGTGTCCCTG CTCAGGTCGA ATCTCAGTCT CCCAACGCCA AGGTCACCTT CTCCAACATC 3180

AAGTTCGGAC CCATTGGCAG CACCGGCAAC CCTAGCGGCG GCAACCCTCC CGGCGGAAAC 3240

CCGCCTGGCA CCACCACCAC CCGCCGCCCA GCCACTACCA CTGGAAGCTC TCCCGGACCT 3300

ACCCAGTCTC ACTACGGCCA GTGCGGCGGT ATTGGCTACA GCGGCCCCAC GGTCTGCGCC 3360

AGCGGCACAA CTTGCCAGGT CCTGAACCCT TACTACTCTC AGTGCCTGTA AAGCTCCGTG 3420

CGAAAGCCTG ACGCACCGGT AGATTCTTGG TGAGCCCGTA TCATGACGGC GGCGGGAGCT 3480

ACATGGCCCC GGGTGATTTA TTTTTTTTGT ATCTACTTCT GACCCTTTTC AAATATACGG 3540

(2) *INFORMATION FOR SEQ ID NO:18:*

(i) SEQUENCE CHARACTERISTICS:
(A) *LENGTH: 2211 base pairs*
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAATTCTCAC GGTGAATGTA GGCCTTTTGT AGGGTAGGAA TTGTCACTCA AGCACCCCCA 60

ACCTCCATTA CGCCTCCCCC ATAGAGTTCC CAATCAGTGA GTCATGGCAC TGTTCTCAAA 120

TAGATTGGGG AGAAGTTGAC TTCCGCCCAG AGCTGAAGGT CGCACAACCG CATGATATAG 180

GGTCGGCAAC GGCAAAAAAG CACGTGGCTC ACCGAAAAGC AAGATGTTTG CGATCTAACA 240

TCCAGGAACC TGGATACATC CATCATCACG CACGACCACT TTGATCTGCT GGTAAACTCG 300

TATTCGCCCT AAACCGAAGT GCGTGGTAAA TCTACACGTG GGCCCCTTTC GGTATACTGC 360

GTGTGTCTTC TCTAGGTGCA TTCTTTCCTT CCTCTAGTGT TGAATTGTTT GTGTTGGGAG 420

TCCGAGCTGT AACTACCTCT GAATCTCTGG AGAATGGTGG ACTAACGACT ACCGTGCACC 480

TGCATCATGT ATATAATAGT GATCCTGAGA AGGGGGGTTT GGAGCAATGT GGGACTTTGA 540

TGGTCATCAA ACAAAGAACG AAGACGCCTC TTTTGCAAAG TTTTGTTTCG GCTACGGTGA 600

AGAACTGGAT ACTTGTTGTG TCTTCTGTGT ATTTTTGTGG CAACAAGAGG CCAGAGACAA 660

TCTATTCAAA CACCAAGCTT GCTCTTTTGA GCTACAAGAA CCTGTGGGGT ATATATCTAG 720

AGTTGTGAAG TCGGTAATCC CGCTGTATAG TAATACGAGT CGCATCTAAA TACTCCGAAG 780

CTGCTGCGAA CCCGGAGAAT CGAGATGTGC TGGAAAGCTT CTAGCGAGCG GCTAAATTAG 840

CATGAAAGGC TATGAGAAAT TCTGGAGACG GCTTGTTGAA TCATGGCGTT CCATTCTTCG 900

ACAAGCAAAG CGTTCCGTCG CAGTAGCAGG CACTCATTCC CGAAAAAACT CGGAGATTCC 960

TAAGTAGCGA TGGAACCGGA ATAATATAAT AGGCAATACA TTGAGTTGCC TCGACGGTTG 1020

CAATGCAGGG GTACTGAGCT TGGACATAAC TGTTCCGTAC CCCACCTCTT CTCAACCTTT 1080

GGCGTTTCCC TGATTCAGCG TACCCGTACA AGTCGTAATC ACTATTAACC CAGACTGACC 1140

GGACGTGTTT TGCCCTTCAT TTGGAGAAAT AATGTCATTG CGATGTGTAA TTTGCCTGCT 1200

TGACCGACTG GGGCTGTTCG AAGCCCGAAT GTAGGATTGT TATCCGAACT CTGCTCGTAG 1260

AGGCATGTTG TGAATCTGTG TCGGGCAGGA CACGCCTCGA AGGTTCACGG CAAGGGAAAC 1320

CACCGATAGC AGTGTCTAGT AGCAACCTGT AAAGCCGCAA TGCAGCATCA CTGGAAAATA 1380

CAAACCAATG GCTAAAAGTA CATAAGTTAA TGCCTAAAGA AGTCATATAC CAGCGGCTAA 1440

TAATTGTACA ATCAAGTGGC TAAACGTACC GTAATTTGCC AACGCGTTGT GGGGTTGCAG 1500

AAGCAACGGC AAAGCCCACT TCCCACGTTT GTTTCTTCAC TCAGTCCAAT CTCAGCTGGT 1560

GATCCCCCAA TTGGGTCGCT TGTTTGTTCC GGTGAAGTGA AAGAAGACAG AGGTAAGAAT 1620

GTCTGACTCG GAGCGTTTTG CATACAACCA AGGGCAGTGA TGGAAGACAG TGAAATGTTG 1680

ACATTCAAGG AGTATTTAGC CAGGGATGCT TGAGTGTATC GTGTAAGGAG GTTTGTCTGC 1740

CGATACGACG AATACTGTAT AGTCACTTCT GATGAAGTGG TCCATATTGA AATGTAAGTC 1800

GGCACTGAAC AGGCAAAAGA TTGAGTTGAA ACTGCCTAAG ATCTCGGGCC CTCGGGCTTC 1860

GGCTTTGGGT GTACATGTTT GTGCTCCGGG CAAATGCAAA GTGTGGTAGG ATCGACACAC 1920

TGCTGCCTTT ACCAAGCAGC TGAGGGTATG TGATAGGCAA ATGTTCAGGG GCCACTGCAT 1980

GGTTTCGAAT AGAAAGAGAA GCTTAGCCAA GAACAATAGC CGATAAAGAT AGCCTCATTA 2040

AACGAAATGA GCTAGTAGGC AAAGTCAGCG AATGTGTATA TATAAAGGTT CGAGGTCCGT 2100

GCCTCCCTCA TGCTCTCCCC ATCTACTCAT CAACTCAGAT CCTCCAGGAG ACTTGTACAC 2160

CATCTTTTGA GGCACAGAAA CCCAATAGTC AACCGCGGAC TGCGCATCAT G 2211

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1137 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAATTCTCAC GGTGAATGTA GGCCTTTTGT AGGGTAGGAA TTGTCACTCA AGCACCCCCA 60

ACCTCCATTA CGCCTCCCCC ATAGAGTTCC CAATCAGTGA GTCATGGCAC TGTTCTCAAA 120

TAGATTGGGG AGAAGTTGAC TTCCGCCCAG AGCTGAAGGT CGCACAACCG CATGATATAG 180

GGTCGGCAAC GGCAAAAAAG CACGTGGCTC ACCGAAAAGC AAGATGTTTG CGATCTAACA 240

TCCAGGAACC TGGATACATC CATCATCACG CACGACCACT TTGATCTGCT GGTAAACTCG 300

TATTCGCCCT AAACCGAAGT GCGTGGTAAA TCTACACGTG GGCCCCTTTC GGTATACTGC 360

GTGTGTCTTC TCTAGGTGCA TTCTTTCCTT CCTCTAGTGT TGAATTGTTT GTGTTGGGAG 420

TCCGAGCTGT AACTACCTCT GAATCTCTGG AGAATGGTGG ACTAACGACT ACCGTGCACC 480

TGCATCATGT ATATAATAGT GATCCTGAGA AGGGGGGTTT GGAGCAATGT GGGACTTTGA 540

TGGTCATCAA ACAAAGAACG AAGACGCCTC TTTTGCAAAG TTTTGTTTCG GCTACGGTGA 600

AGAACTGGAT ACTTGTTGTG TCTTCTGTGT ATTTTTGTGG CAACAAGAGG CCAGAGACAA 660

TCTATTCAAA CACCAAGCTT GCTCTTTTGA GCTACAAGAA CCTGTGGGGT ATATATCTAG 720

TGGCCAGAAT GCCTAGGTCA CCTCTAGAGA GTTGAAACTG CCTAAGATCT CGGGCCCTCG 780

GGCTTCGGCT TTGGGTGTAC ATGTTTGTGC TCCGGGCAAA TGCAAAGTGT GGTAGGATCG 840

ACACACTGCT GCCTTTACCA AGCAGCTGAG GGTATGTGAT AGGCAAATGT TCAGGGGCCA 900

CTGCATGGTT TCGAATAGAA AGAGAAGCTT AGCCAAGAAC AATAGCCGAT AAAGATAGCC 960

TCATTAAACG AAATGAGCTA GTAGGCAAAG TCAGCGAATG TGTATATATA AAGGTTCGAG 1020

GTCCGTGCCT CCCTCATGCT CTCCCCATCT ACTCATCAAC TCAGATCCTC CAGGAGACTT 1080

GTACACCATC TTTTGAGGCA CAGAAACCCA ATAGTCAACC GCGGACTGCG CATCATG 1137

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2261 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GAATTCTCAC GGTGAATGTA GGCCTTTTGT AGGGTAGGAA TTGTCACTCA AGCACCCCCA 60

ACCTCCATTA CGCCTCCCCC ATAGAGTTCC CAATCAGTGA GTCATGGCAC TGTTCTCAAA 120

TAGATTGGGG AGAAGTTGAC TTCCGCCCAG AGCTGAAGGT CGCACAACCG CATGATATAG 180

GGTCGGCAAC GGCAAAAAAG CACGTGGCTC ACCGAAAAGC AAGATGTTTG CGATCTAACA 240

TCCAGGAACC TGGATACATC CATCATCACG CACGACCACT TTGATCTGCT GGTAAACTCG 300

TATTCGCCCT AAACCGAAGT GCGTGGTAAA TCTACACGTG GGCCCCTTTC GGTATACTGC 360

GTGTGTCTTC TCTAGGTGCA TTCTTTCCTT CCTCTAGTGT TGAATTGTTT GTGTTGGGAG 420

TCCGAGCTGT AACTACCTCT GAATCTCTGG AGAATGGTGG ACTAACGACT ACCGTGCACC 480

TGCATCATGT ATATAATAGT GATCCTGAGA AGGGGGGTTT GGAGCAATGT GGGACTTTGA 540

TGGTCATCAA ACAAAGAACG AAGACGCCTC TTTTGCAAAG TTTTGTTTCG GCTACGGTGA 600

AGAACTGGAT ACTTGTTGTG TCTTCTGTGT ATTTTTGTGG CAACAAGAGG CCAGAGACAA 660

TCTATTCAAA CACCAAGCTT GCTCTTTTGA GCTACAAGAA CCTGTGGGGT ATATATCTAG 720

TGGCCAGAAT GCCTAGGTCA CCTCTAAAGG TACCCTGCAG CTCGAGCTAG AGTTGTGAAG 780

TCGGTAATCC CGCTGTATAG TAATACGAGT CGCATCTAAA TACTCCGAAG CTGCTGCGAA 840

CCCGGAGAAT CGAGATGTGC TGGAAAGCTT CTAGCGAGCG GCTAAATTAG CATGAAAGGC 900

TATGAGAAAT TCTGGAGACG GCTTGTTGAA TCATGGCGTT CCATTCTTCG ACAAGCAAAG 960

CGTTCCGTCG CAGTAGCAGG CACTCATTCC CGAAAAAACT CGGAGATTCC TAAGTAGCGA 1020

TGGAACCGGA ATAATATAAT AGGCAATACA TTGAGTTGCC TCGACGGTTG CAATGCAGGG 1080

GTACTGAGCT TGGACATAAC TGTTCCGTAC CCCACCTCTT CTCAACCTTT GGCGTTTCCC 1140

TGATTCAGCG TACCCGTACA AGTCGTAATC ACTATTAACC CAGACTGACC GGACGTGTTT 1200

TGCCCTTCAT TTGGAGAAAT AATGTCATTG CGATGTGTAA TTTGCCTGCT TGACCGACTG 1260

GGGCTGTTCG AAGCCCGAAT GTAGGATTGT TATCCGAACT CTGCTCGTAG AGGCATGTTG 1320

TGAATCTGTG TCGGGCAGGA CACGCCTCGA AGGTTCACGG CAAGGGAAAC CACCGATAGC 1380

AGTGTCTAGT AGCAACCTGT AAAGCCGCAA TGCAGCATCA CTGGAAAATA CAAACCAATG 1440

GCTAAAAGTA CATAAGTTAA TGCCTAAAGA AGTCATATAC CAGCGGCTAA TAATTGTACA 1500

ATCAAGTGGC TAAACGTACC GTAATTTGCC AACGCGTTTC TAGATTGCAG AAGCACGGCA 1560

AAGCCCACTT ACCCACGTTT GTTTCTTCAC TCAGTCCAAT CTCAGCTGGT GATCCCCCAA 1620

TTGGGTCGCT TGTTTGTTCC GGTGAAGTGA AAGAAGACAG AGGTAAGAAT GTCTGACTCG 1680

GAGCGTTTTG CATACAACCA AGGGCAGTGA TGGAAGACAG TGAAATGTTG ACATTCAAGG 1740

AGTATTTAGC CAGGGATGCT TGAGTGTATC GTGTAAGGAG GTTTGTCTGC CGATACGACG 1800

AATACTGTAT AGTCACTTCT GATGAAGTGG TCCATATTGA AATGTAAGTC GGCACTGAAC 1860

AGGCAAAAGA TTGAGTTGAA ACTGCCTAAG ATCTCGGGCC CTCGGGCTTC GGCTTTGGGT 1920

GTACATGTTT GTGCTCCGGG CAAATGCAAA GTGTGGTAGG ATCGACACAC TGCTGCCTTT 1980

ACCAAGCAGC TGAGGGTATG TGATAGGCAA ATGTTCAGGG GCCACTGCAT GGTTTCGAAT 2040

AGAAAGAGAA GCTTAGCCAA GAACAATAGC CGATAAAGAT AGCCTCATTA AACGAAATGA 2100

GCTAGTAGGC AAAGTCAGCG AATGTGTATA TATAAAGGTT CGAGGTCCGT GCCTCCCTCA 2160

TGCTCTCCCC ATCTACTCAT CAACTCAGAT CCTCCAGGAG ACTTGTACAC CATCTTTTGA 2220

GGCACAGAAA CCCAATAGTC AACCGCGGAC TGCGCATCAT G 2261

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1776 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAATTCTCAC GGTGAATGTA GGCCTTTTGT AGGGTAGGAA TTGTCACTCA AGCACCCCCA 60

ACCTCCATTA CGCCTCCCCC ATAGAGTTCC CAATCAGTGA GTCATGGCAC TGTTCTCAAA 120

TAGATTGGGG AGAAGTTGAC TTCCGCCCAG AGCTGAAGGT CGCACAACCG CATGATATAG 180

GGTCGGCAAC GGCAAAAAAG CACGTGGCTC ACCGAAAAGC AAGATGTTTG CGATCTAACA 240

TCCAGGAACC TGGATACATC CATCATCACG CACGACCACT TTGATCTGCT GGTAAACTCG 300

TATTCGCCCT AAACCGAAGT GCGTGGTAAA TCTACACGTG GGCCCCTTTC GGTATACTGC 360

GTGTGTCTTC TCTAGGTGCA TTCTTTCCTT CCTCTAGTGT TGAATTGTTT GTGTTGGGAG 420

TCCGAGCTGT AACTACCTCT GAATCTCTGG AGAATGGTGG ACTAACGACT ACCGTGCACC 480

TGCATCATGT ATATAATAGT GATCCTGAGA AGGGGGGTTT GGAGCAATGT GGGACTTTGA 540

TGGTCATCAA ACAAAGAACG AAGACGCCTC TTTTGCAAAG TTTTGTTTCG GCTACGGTGA 600

AGAACTGGAT ACTTGTTGTG TCTTCTGTGT ATTTTTGTGG CAACAAGAGG CCAGAGACAA 660

TCTATTCAAA CACCAAGCTT GCTCTTTTGA GCTACAAGAA CCTTCTAAAT ATATATCTAG 720

TGGCCAGAAT GCCTAGGTCA CCTCTAAATG TGTAATTTGC CTGCTTGACC GACTGGGGCT 780

GTTCGAAGCC CGAATGTAGG ATTGTTATCC GAACTCTGCT CGTAGAGGCA TGTTGTGAAT 840

CTGTGTCGGG CAGGACACGC CTCGAAGGTT CACGGCAAGG GAAACCACCG ATAGCAGTGT 900

CTAGTAGCAA CCTGTAAAGC CGCAATGCAG CATCACTGGA AAATACAAAC CAATGGCTAA 960

AAGTACATAA GTTAATGCCT AAAGAAGTCA TATACCAGCG GCTAATAATT GTACAATCAA 1020

GTGGCTAAAC GTACCGTAAT TTGCCAACGC GTTTCTAGAT TGCAGAAGCA CGGCAAAGCC 1080

CACTTACCCA CGTTTGTTTC TTCACTCAGT CCAATCTCAG CTGGTGATCC CCCAATTGGG 1140

TCGCTTGTTT GTTCCGGTGA AGTGAAAGAA GACAGAGGTA AGAATGTCTG ACTCGGAGCG 1200

TTTTGCATAC AACCAAGGGC AGTGATGGAA GACAGTGAAA TGTTGACATT CAAGGAGTAT 1260

TTAGCCAGGG ATGCTTGAGT GTATCGTGTA AGGAGGTTTG TCTGCCGATA CGACGAATAC 1320

TGTATAGTCA CTTCTGATGA AGTGGTCCAT ATTGAAATGT AAGTCGGCAC TGAACAGGCA 1380

AAAGATTGAG TTGAAACTGC CTAAGATCTC GGGCCCTCGG GCTTCGGCTT TGGGTGTACA 1440

TGTTTGTGCT CCGGGCAAAT GCAAAGTGTG GTAGGATCGA CACACTGCTG CCTTTACCAA 1500

GCAGCTGAGG GTATGTGATA GGCAAATGTT CAGGGGCCAC TGCATGGTTT CGAATAGAAA 1560

GAGAAGCTTA GCCAAGAACA ATAGCCGATA AAGATAGCCT CATTAAACGA AATGAGCTAG 1620

TAGGCAAAGT CAGCGAATGT GTATATATAA AGGTTCGAGG TCCGTGCCTC CCTCATGCTC 1680

TCCCCATCTA CTCATCAACT CAGATCCTCC AGGAGACTTG TACACCATCT TTTGAGGCAC 1740

AGAAACCCAA TAGTCAACCG CGGACTGCGC ATCATG 1776

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1776 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CAATTCTCAC GGTGAATGTA GGCCTTTTGT AGGGTAGGAA TTGTCACTCA AGCACCCCCA 60

ACCTCCATTA CGCCTCCCCC ATAGAGTTCC CAATCAGTGA GTCATGGCAC TGTTCTCAAA 120

TAGATTGGGG AGAAGTTGAC TTCCGCCCAG AGCTGAAGGT CGCACAACCG CATGATATAG 180

GGTCGGCAAC GGCAAAAAAG CACGTGGCTC ACCGAAAAGC AAGATGTTTG CGATCTAACA 240

TCCAGGAACC TGGATACATC CATCATCACG CACGACCACT TTGATCTGCT GGTAAACTCG 300

TATTCGCCCT AAACCGAAGT GCGTGGTAAA TCTACACGTG GGCCCCTTTC GGTATACTGC 360

GTGTGTCTTC TCTAGGTGCA TTCTTTCCTT CCTCTAGTGT TGAATTGTTT GTGTTGGGAG 420

TCCGAGCTGT AACTACCTCT GAATCTCTGG AGAATGGTGG ACTAACGACT ACCGTGCACC 480

TGCATCATGT ATATAATAGT GATCCTGAGA AGGGGGGTTT GGAGCAATGT GGGACTTTGA 540

TGGTCATCAA ACAAAGAACG AAGACGCCTC TTTTGCAAAG TTTTGTTTCG GCTACGGTGA 600

AGAACTGGAT ACTTGTTGTG TCTTCTGTGT ATTTTTGTGG CAACAAGAGG CCAGAGACAA 660

TCTATTCAAA CACCAAGCTT GCTCTTTTGA GCTACAAGAA CCTTCTAAAT ATATATCTAG 720

TGGCCAGAAT GCCTAGGTCA CCTCTAAATG TGTAATTTGC CTGCTTGACC GATCTAAACT 780

GTTCGAAGCC CGAATGTAGG ATTGTTATCC GAACTCTGCT CGTAGAGGCA TGTTGTGAAT 840

CTGTGTCGGG CAGGACACGC CTCGAAGGTT CACGGCAAGG GAAACCACCG ATAGCAGTGT 900

CTAGTAGCAA CCTGTAAAGC CGCAATGCAG CATCACTGGA AAATACAAAC CAATGGCTAA 960

AAGTACATAA GTTAATGCCT AAAGAAGTCA TATACCAGCG GCTAATAATT GTACAATCAA 1020

GTGGCTAAAC GTACCGTAAT TTGCCAACGC GTTTCTAGAT TGCAGAAGCA CGGCAAAGCC 1080

CACTTACCCA CGTTTGTTTC TTCACTCAGT CCAATCTCAG CTGGTGATCC CCCAATTGGG 1140

TCGCTTGTTT GTTCCGGTGA AGTGAAAGAA GACAGAGGTA AGAATGTCTG ACTCGGAGCG 1200

TTTTGCATAC AACCAAGGGC AGTGATGGAA GACAGTGAAA TGTTGACATT CAAGGAGTAT 1260

TTAGCCAGGG ATGCTTGAGT GTATCGTGTA AGGAGGTTTG TCTGCCGATA CGACGAATAC 1320

TGTATAGTCA CTTCTGATGA AGTGGTCCAT ATTGAAATGT AAGTCGGCAC TGAACAGGCA 1380

AAAGATTGAG TTGAAACTGC CTAAGATCTC GGGCCCTCGG GCTTCGGCTT TGGGTGTACA 1440

TGTTTGTGCT CCGGGCAAAT GCAAAGTGTG GTAGGATCGA CACACTGCTG CCTTTACCAA 1500

GCAGCTGAGG GTATGTGATA GGCAAATGTT CAGGGGCCAC TGCATGGTTT CGAATAGAAA 1560

GAGAAGCTTA GCCAAGAACA ATAGCCGATA AAGATAGCCT CATTAAACGA AATGAGCTAG 1620

TAGGCAAAGT CAGCGAATGT GTATATATAA AGGTTCGAGG TCCGTGCCTC CCTCATGCTC 1680

TCCCCATCTA CTCATCAACT CAGATCCTCC AGGAGACTTG TACACCATCT TTTGAGGCAC 1740

AGAAACCCAA TAGTCAACCG CGGACTGCGC ATCATG 1776

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 745 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGACCTACCC AGTCTCACTA CGGCCAGTGC GGCGGTATTG GCTACAGCGG CCCCACGGTC 60

TGCGCCAGCG GCACAACTTG CCAGGTCCTG AACCCTTACT ACTCTCAGTG CCTGTAAAGC 120

TCCGTGCGAA AGCCTGACGC ACCGGTAGAT TCTTGGTGAG CCCGTATCAT GACGGCGGCG 180

GGAGCTACAT GGCCCCGGGT GATTTATTTT TTTTGTATCT ACTTCTGACC CTTTTCAAAT 240

ATACGGTCAA CTCATCTTTC ACTGGAGATG CGGCCTGCTT GGTATTGCGA TGTTGTCAGC 300

TTGGCAAATT GTGGCTTTCG AAAACACAAA ACGATTCCTT AGTAGCCATG CATTTTAAGA 360

TAACGGAATA GAAGAAAGAG GAAATTAAAA AAAAAAAAAA AACAAACATC CCGTTCATAA 420

CCCGTAGAAT CGCCGCTCTT CGTGTATCCC AGTACCACGT CAAAGGTATT CATGATCGTT 480

CAATGTTGAT ATTGTTCCGC CAGTATGGCT CCACCCCCAT CTCCGCGAAT CTCCTCTTCT 540

CGAACGCGGT AGTGGCTGCT GCCAATTGGT AATGACCATA GGGAGACAAA CAGCATAATA 600

GCAACAGTGG AAATTAGTGG CGCAATAATT GAGAACACAG TGAGACCATA GCTGGCGGCC 660

TGGAAAGCAC TGTTGGAGAC CAACTTGTCC GTTGCGAGGC CAACTTGCAT TGCTGTCAAG 720

ACGATGACAA CGTAGCCGAG GACCC 745

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1627 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGCGGTATTG GCTACAGCGG CCCCACGGTC TGCGCCAGCG GCACAACTTG CCAGGTCCTG 60

AACCCTTACT ACTCTCAGTG CCTGTAAAGC TCCGTGCGAA AGCCTGACGC ACCGGTAGAT 120

TCTTGGTGAG CCCGTATCAT GACGGCGGCG GGAGCTACAT GGCCCCGGGT GATTTATTTT 180

TTTTGTATCT ACTTCTGACC CTTTTCAAAT ATACGGTCAA CTCATCTTTC ACTGGAGATG 240

CGGCCTGCTT GGTATTGCGA TGTTGTCAGC TTGGCAAATT GTGGCTTTCG AAAACACAAA 300

ACGATTCCTT AGTAGCCATG CATCGGGATC CTTTAAGATA ACGGAATAGA AGAAAGAGGA 360

AATTAAAAAA AAAAAAAAAA CAAACATCCC GTTCATAACC CGTAGAATCG CCGCTCTTCG 420

TGTATCCCAG TACCACGGCA AAGGTATTTC ATGATCGTTC AATGTTGATA TTGTTCCCGC 480

CAGTATGGCT GCACCCCCAT CTCCGCGAAT CTCCTCTTCT CGAACGCGGT AGTGGCGCGC 540

CAATTGGTAA TGACCATAGG GAGACAAACA GCATAATAGC AACAGTGGAA ATTAGTGGCG 600

CAATAATTGA GAACACAGTG AGACCATAGC TGGCGGCCTG GAAAGCACTG TTGGAGACCA 660

ACTTGTCCGT TGCGAGGCCA ACTTGCATTG CTGTCAAGAC GATGACAACG TAGCCGAGGA 720

CCGTCACAAG GGACGCAAAG TTGTCGCGGA TGAGGTCTCC GTAGATGGCA TAGCCGGCAA 780

TCCGAGAGTA GCCTCTCAAC AGGTGGCCTT TTCGAAACCG GTAAACCTTG TTCAGACGTC 840

CTAGCCGCAG CTCACCGTAC CAGTATCGAG GATTGACGGC AGAATAGCAG TGGCTCTCCA 900

GGATTTGACT GGACAAAATC TTCCAGTATT CCCAGGTCAC AGTGTCTGGC AGAAGTCCCT 960

TCTCGCGTGC ANTCGAAAGT CGCTATAGTG CGCAATGAGA GCACAGTAGG AGAATAGGAA 1020

CCCGCGAGCA CATTGTTCAA TCTCCACATG AATTGGATGA CTGCTGGGCA GAATGTGCTG 1080

CCTCCAAAAT CCTGCGTCCA ACAGATACTC TGGCAGGGGC TTCAGATGAA TGCCTCTGGG 1140

CCCCCAGATA AGATGCAGCT CTGGATTCTC GGTTACNATG ATATCGCGAG AGAGCACGAG 1200

TTGGTGATGG AGGGACAGGA GGCATAGGTC GCGCAGGCCC ATAACCAGTC TTGCACAGCA 1260

TTGATCTTAC CTCACGAGGA GCTCCTGATG CAGAAACTCC TCCATGTTGC TGATTGGGTT 1320

GAGAATTTCA TCGCTCCTGG ATCGTATGGT TGCTGGCAAG ACCCTGCTTA ACCGTGCCGT 1380

GTCATGGTCA TCTCTGGTGG CTTCGTCGCT GGCCTGTCTT TGCAATTCGA CAGCAAATGG 1440

TGGAGATCTC TCTATCGTGA CAGTCATGGT AGCGATAGCT AGGTGTCGTT GCACGCACAT 1500

AGGCCGAAAT GCGAAGTGGA AAGAATTTCC CGGNTGCGGA ATGAAGTCTC GTCATTTTGT 1560

ACTCGTACTC GACACCTCCA CCGAAGTGTT AATAATGGAT CCACGATGCC AAAAAGCTTG 1620

TGCATGC 1627

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 91 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
GGACTGGCAT CATGGCGCCC TCAGTTACAC TGCCGTTGAC CACGGCCATC CTGGCCATTG      60

CCCGGCTCGT CGCCGCCCAG CAACCGGGTA C                                     91
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 18..95

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
AACCGCGGAC TGGCATC ATG GCG CCC TCA GTT ACA CTG CCG TTG ACC ACG         50
                   Met Ala Pro Ser Val Thr Leu Pro Leu Thr Thr
                     1               5                  10

GCC ATC CTG GCC ATT GCC CGG CTC GTC GCC GCC CAG CAA CCG GGT           95
Ala Ile Leu Ala Ile Ala Arg Leu Val Ala Ala Gln Gln Pro Gly
            15                  20                  25

AC                                                                    97
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Met Ala Pro Ser Val Thr Leu Pro Leu Thr Thr Ala Ile Leu Ala Ile
 1               5                  10                  15

Ala Arg Leu Val Ala Ala Gln Gln Pro Gly
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
ACT ACG TAG TCG ACT                                                   15
```

WHAT IS CLAIMED IS:

1. A method for cloning a promoter that is active in a desired environmental condition, said method comprising:
   a. exposing a host to said environmental condition;
   b. extracting mRNA from said host;
   c. preparing a cDNA bank from said mRNA;
   d. detectably labelling a sample of said cDNA;
   e. hybridizing said labelled labelled cDNA to said cDNA bank;
   f. selecting clones from said hybridization of step (e) on the basis of the intensity of the hybridization;
   g. determining the relative abundancy of said selected clones in the cDNA bank of step (c);
   h. identifying the most abundant clones of step (g); and
   i. using the inserts of the clones of step (h) to identify and clone the host promoter that was responsible for expression of the corresponding mRNA under said environmental condition.

2. The method of claim 1, wherein said condition is growth in glucose-containing medium.

3. The method of claim 1, wherein the host is a filamentous fungi.

4. The method of claim 1, wherein the host is selected from the group consisting of *Trichoderma*, *Aspergillus*, *Claviceps purpurea*, *Penicillium chrysogenum*, *Magnaporthe grisea*, *Neurospora*, *Mycosphaerella spp.*, *Collectotrichum trifolii*, the dimorphic fungus *Histoplasmia capsulatum*, *Nectia haematococca* (anamorph:*Fusarium solani* f. sp. *phaseoli* and f. sp. *pisi*), *Ustilago violacea*, *Ustilago maydis*, *Cephalosporium acremonium*, *Schizophyllum commune*, *Podospora anserina*, *Sordaria macrospora*, *Mucor circinelloides*, and *Collectotrichum capsici*.

5. The method of claim 4, wherein the host is *Trichoderma*.

6. The method of claim 5, wherein the host is *T. reesei*.

7. An isolated promoter capable of expression of an operably-linked coding sequence in a fungal host grown on glucose.

8. The promoter of claim 7, wherein said promoter is cloned by a method comprising:

a. exposing a host to said environmental condition;

b. extracting mRNA from said host;

c. preparing a cDNA bank from a first sample of said mRNA;

d. detectably labelling a sample of said cDNA;

e. hybridizing said labelled labelled cDNA to said cDNA bank;

f. selecting clones from said hybridization of step (e) on the basis of the intensity of the hybridization;

g. determining the relative abundancy of said selected clones in the cDNA bank of step (c);

h. identifying the most abundant clones of step (g); and

i. using the inserts of the clones of step (h) to identify and clone the host promoter that was responsible for expression of the corresponding mRNA under said environmental condition.

9. The promoter of claim 7, wherein said host is a filamentous fungi.

10. The promoter of claim 9, wherein said host is selected from the group consisting of *Trichoderma*, *Aspergillus*, *Claviceps purpurea*, *Penicillium chrysogenum*, *Magnaporthe grisea*, *Neurospora*, *Mycosphaerella spp.*, *Collectotrichum trifolii*, the dimorphic fungus *Histoplasmia capsulatum*, *Nectia haematococca* (anamorph:*Fusarium solani* f. sp. *phaseoli* and f. sp. *pisi*), *Ustilago violacea*, *Ustilago maydis*, *Cephalosporium acremonium*, *Schizophyllum commune*, *Podospora anserina*, *Sordaria macrospora*, *Mucor circinelloides*, and *Collectotrichum capsici*.

11. The promoter of claim 10, wherein said host is *Trichoderma*.

12. The promoter of claim 11, wherein said host is selected from the group consisting of *T. reesei*, *T. harzianum*, *T. longibrachiatum*, *T. viride*, and *T. koningii*.

13. The promoter of claim 12, wherein said host is *T. reesei*.

14. The promoter of claim 13, wherein said promoter is the *tef1* promoter.

15. The promoter of claim 14, wherein said *tef1* promoter contains promoter elements of the 1.2 kb sequence adjacent to the translational start site of SEQ ID 1.

16. The promoter of claim 13, wherein said promoter is the promoter of SEQ ID 2.

17. The promoter of claim 13, wherein said promoter is the promoter of SEQ ID 3.

18. The promoter of claim 13, wherein said promoter is the promoter of SEQ ID 4.

19. The promoter of claim 13, wherein said promoter is the promoter of SEQ ID 5.

20. The promoter of claim 13, wherein said promoter is the promoter of SEQ ID 6.

21. The promoter of claim 7, wherein said promoter is an altered *cbh1* promoter, such alteration decreasing the ability of glucose to repress said *cbh1* promoter.

22. The promoter of claim 21, wherein said native *cbh1* promoter has an altered mig-like sequence at approximately position -720 to -715.

23. The promoter of claim 22, wherein said mig-like sequence is 5'-GTGGGG.

24. The promoter of claim 22, wherein said altered mig-like sequence 5'-TCTAGA.

25. The promoter of claim 24, wherein said promoter is the *cbh1* promoter of pMI-24.

26. The promoter of claim 21, wherein said native *cbh1* promoter has the sequence TCTAAA at position -1505 to -1500 and the sequence TCTAGA at position -720 to -715.

27. The promoter of claim 22, wherein said native *cbh1* promoter has the sequence TCTAAA at position -1505 to -1500 and the sequence TCTAAA at position -1001 to -996 and the sequence TCTAGA at position -720 to -715.

28. A promoter, wherein said promoter is selected from the *cbh1* promoter of the group consistin of pML016del5(11), pMI-24, pMI-27, pMI-28, pMLO16del5(11), SEQ ID 19, SEQ ID 20, SEQ ID 21 and SEQ ID 22.

29. A vector comprising the promoter of claim 7.

30. The vector of claim 29, wherein said promoter is operably linked to a coding sequence.

31. The vector of claim 30, wherein said coding sequence encodes an enzyme hydrolysing lignocellulose.

32. A host cell transformed with the vector of claim 31.

33. The vector of claim 32, wherein said vector is selected from the group consisting of pTHN100B, pML016del5(11), pMI-24, pMI-27, pMI-28.

34. A host cell transformed with the vector of claim 33.

35. A host cell transformed with the vector of claim 30.

36. The host cell of claim 35, wherein said cell is a fungal cell.

37. The host cell of claim 36, wherein said fungal cell is that of a fungus selected from the group consisting of *Trichoderma*, *Aspergillus*, *Claviceps purpurea*, *Penicillium chrysogenum*, *Magnaporthe grisea*, *Neurospora*, *Mycosphaerella spp.*, *Collectotrichum trifolii*, the dimorphic fungus *Histoplasmia capsulatum*, *Nectia haematococca* (anamorph:*Fusarium solani* f. sp. *phaseoli* and f. sp. *pisi*), *Ustilago violacea*, *Ustilago maydis*, *Cephalosporium acremonium*, *Schizophyllum commune*, *Podospora anserina*, *Sordaria macrospora*, *Mucor circinelloides*, and *Collectotrichum capsici*.

38. The host cell of claim 37, wherein said fungus is *Trichoderma*.

39. The host cell of claim 38, wherein said fungus is selected from the group consisting of *T. reesei*, *T. harzianum*, *T. longibrachiatum*, *T. viride*, and *T. koningii*.

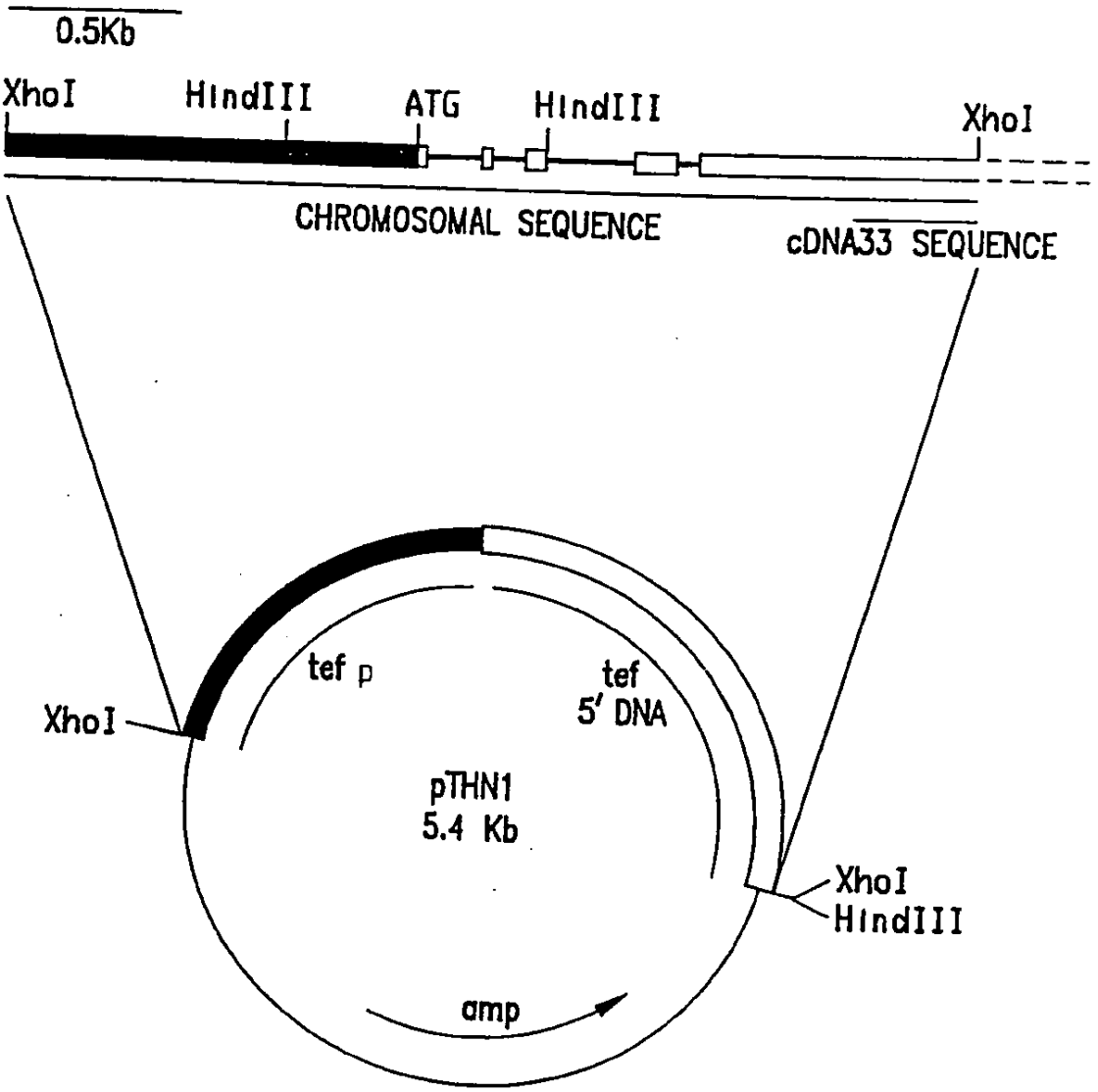
40. The host cell of claim 39, wherein said fungus is *T. reesei*.

41. An enzyme composition produced by a method comprising:

a. growing the host cell of claim 35 in the presence of glucose;

b. separating the host cell from the growth medium; and

c. using said growth medium of step (b) as the source of the enzymes in said enzyme composition.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HindIII
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HindIII
XhoI
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cDNA33 SEQUENCE
XhoI
tef p
tef
5' DNA
pTHN1
5.4 Kb
XhoI
HindIII
amp

FIG.1

```
          10         20         30         40         50         60
   1 CGCCGTGACG ACAGAAACGG AGCCCGCGAG TTTGGATACG CCGCTGAAAT GGGGCTTGAC
  61 GGTGAAGGAG AAGCCGAGCG CGGTGCCAGA GGACAAGATG GATGTAGAGC CAGGCGACGA
 121 CGACCAAACG CAACCATCAA ATCAATCAGA TGGCAATGAC GCACCACCGC CCCAGCAGCG
 181 CGAACCGCCG ACGAAGAAGC CATGGACGCG CTCCTCGGCA AGACGCCCAA GGAACAGAAA
 241 AAAGTAATCT CCGCACCCGT ATCAGAAGAC GACGCCTACC GCCGCGACGT CGAAGCCTCC
 301 GGCGCGGTGT CCACGCTCCA GGATTACGAA GACATGCCCG TCGAGGAGTT TGGCGCCGCC
 361 CTCCTCCNNN GCATGGGCTG GAACGGGGAA GCCCGCGGCC CGCCGGTCAA GCAGGTCAAG
 421 AGGCGGCAGA ACAGGCTCGG CCTCGGCGCC AAGGAGCTCA AGGAGGAAGA GGACCTCGGC
 481 GGGTGGAACC AGAACGGCAA GAAAAAGTCG AGGCCSCGCG GCTGAGCGAG TATCGGAGGG
 541 AGGAGAGCAA GCGCAAGGAA GGCCGGGGGC ATGAGGACAG CTATAAACGA GAGAGGGAGC
 601 GCGAACGGAT CGCGAGAGGG ATCACTACAG GGAGCGAGAC CGGGACAGGG ATCGCGATTA
 661 TAGGGATCGG GATAGGGATA GACATCGGGA CCACGATAGG CACAGGGACC GACATCGCGA
 721 CTCTGACCGG CACCATCGAC GATGAAGGAG CTTTTGCATT CTTCTCTTCG TCAACCACTT
 781 TTGAGACTAA CATTAACCAT GCCGTTTTCT TGAAAAGCTT GTACTCATCA TGATGTTTTT
 841 AAGCAAATAG GCGACAGGCG TACAGACACC TTAATATCAC ATAGAGGCAC GGCACACATA
 901 CGTCTTGGAG AAGACACGTA CTTACGAATG ATGGGAGAAT TACCTACTCT GACTTGTGTA
 961 AATTAGAATA TCAATGACAC TATGTATATT CAGTCGAGCT GCGAATGGTC ACACATTGTC
1021 TGATCTGCGA ATTTGTATGT GCTGCCTCTC CCTCTGACCT TCTGGTCTGG TGATACCATC
1081 CTCCCTCAGT TTGGATCATC GCCTTATTCT TCTTCCCTCT TCTGCATCTG CTTCCTGCTC
1141 GTTTGAGGAA CATCGCCAGC TGACTCTGCT TGCCTCGCAG CGATCTAGTC AAGAACAACA
1201 CNAGCTCTCA CGCTACATCA CACAAACCGT CAAAATGGGT AAGGAGGACA AGACTCACAT
1261 CAACGTGGTC GTCATCGTAC GTATTTTCCG ATCCCTCATC GGCNGTCATC TGNCCAGTCT
1321 GATTCCAAGA ATCACCGTGC TAACCATATA CCATCTANGG GTGCGTATTC CATCAATCAT
1381 CTTGAGCCAG ATCGACCGAA CATACGATAC TGACTTTGCT ACGACAGCCA CGTCGACTCC
```

FIG.1A-1

1441 GGCAAGTCTA CCACCGTGAG TAAACACCCA TTCCACTCCA CGACCGCAAG CTCCATCTTG
1501 CGCGTGGCGT CTCTGCGATG AACATCCGAA ACTGACGTTC TGTTACAGAC TGGTCACTTG
1561 ATCTACCAGT GCGGTGGTAT CGACAAGCGT ACCATTGAGA AGTTCGAGAA GGTAAGCTTC
1621 GTTCCTTAAA TCTCCAGACG CGAGCCCAAT CTTTGCCCAT CTGCCCAGCA TCTGGCGAAC
1681 GAATGCTGTG CCGACACGAT TTTTTTTTTC ATCACCCCGC TTTCTCCTAC CCCTCCTTCG
1741 AGCGACGCAA ATTTTTTTTG CTGCCTTACG AGTTTTAGTG GGGTCGCACC TCACAACCCC
1801 ACTACTGCTC TCTGGCCGCT CCCCAGTCAC CCAACGTCAT CAACGCAGCA GTTTTCAATC
1861 AGCGATGCTA ACCATATTCC CTCGAACAGG AAGCCGCCGA ACTCGGCAAG GGTTCCTTCA
1921 AGTACGCGTG GGTTCTTGAC AAGCTCAAGG CCGAGCGTGA GCGTGGTATC ACCATCGACA
1981 TTGCCCTCTG GAAGTTCGAG ACTCCCAAGT ACTATGTCAC CGTCATTGGT ATGTTGGCAG
2041 CCATCACCTC ACTGCGTCGT TGACACATCA AACTAACAAT GCCCTCACAG ACGCTCCCGG
2101 CCACCGTGAC TTCATCAAGA ACATGATCAC TGGTACTTCC CAGGCCGACT GCGCTATCCT
2161 CATCATCGCT GCCGGTACTG GTGAGTTCGA GGCTGGTATC TCCAAGGATG GCCAGACCCG
2221 TGAGCACGCT CTGCTCGCCT ACACCCTGGG TGTCAAGCAG CTCATCGTCG CCATCAACAA
2281 GATGGACACT GCCAACTGGG CCGAGGCTCG TTACCAGGAA ATCATCAAGG AGACTTCCAA
2341 CTTCATCAAG AAGGTCGGCT TCAACCCCAA GGCCGTTGCT TTCGTCCCCA TCTCCGGCTT
2401 CAACGGTGAC AACATGCTCA CCCCCTCCAC CAACTGCCCC TGGTACAAGG GCTGGGAGAA
2461 GGAGACCAAG GCTGGCAAGT TCACCGGCAA GACCCTCCTT GAGGCCATCG ACTCCATCGA
2521 GCCCCCCAAG CGTCCCACGG ACAAGCCCCT GCGTCTTCCC CTCCAGGACG TCTACAAGAT
2581 CGGTGGTATC GGAACAGTTC CCGTCGGCCG TATCGAGACT GGTGTCCTCA AGCCCGGTAT
2641 GGTCGTTACC TTCGCTCCCT CCAACGTCAC CACTGAAGTC AAGTCCGTCG AGATGCACCA
2701 CGAGCAGCTC GCTGAGGGCC AGCCTGGTGA CAACGTTGGT TTCAACGTGA AGAACGTTTC
2761 CGTCAAGGAA ATCCGCCGTG GCAACGTTGC CGGTGACTCC AAGAACGACC CCCCCATGGG

FIG.1A-2

2821 CGCCGCTTCT TTCACCGCCC AGGTCATCGT CATGAACCAC CCCGGCCAGG TCGGTGCCGG
2881 CTACGCCCCC GTCCTCGACT GCCACACTGC CCACATTGCC TGCAAGTTCG CCGAGCTCCT
2941 CGAGAAGATC GACCGCCGTA CCGGTAAGGC TACCGAGTCT GCCCCCAAGT TCATCAAGTC
3001 TGGTGACTCC GCCATCGTCA AGATGATCCC CTCCAAGCCC ATGTGCGTTG AGGCTTTCAC
3061 CGACTACCCT CCCCTGGGTC GTTTCGCCGT CCGTGACATG CGCCAGACCG TCGCTGTCGG
3121 TGTCATCAAG GCCGTCGAGA AGTCCTCTGC CGCCGCCGCN AAGGTCACCA AGTCCGCTGC
3181 CAAGGCCGCC AAGAAATAAG CGATACCCAT CATCAACACC TGATGTTCTG GGGTCCCTCG
3241 TGAGGTTTCT CCAGGTGGGC ACCACCATGC GCTCACTTCT ACGACGAAAC GATCAATGTT
3301 GCTATGCATG AGSACTCGAC TATGAATCGA GGCACGGTTA ATTGAGAGGC TGGGAATAAG
3361 GGTTCCATCA GAACTTCTCT GGGAATGCAA AACAAAAGGG AACAAAAAAA CTAGATAGAA
3421 GTGAATTCAT GACTTCGACA ACCAAAAAAA AAAAAAAAAA A

FIG.1A-3

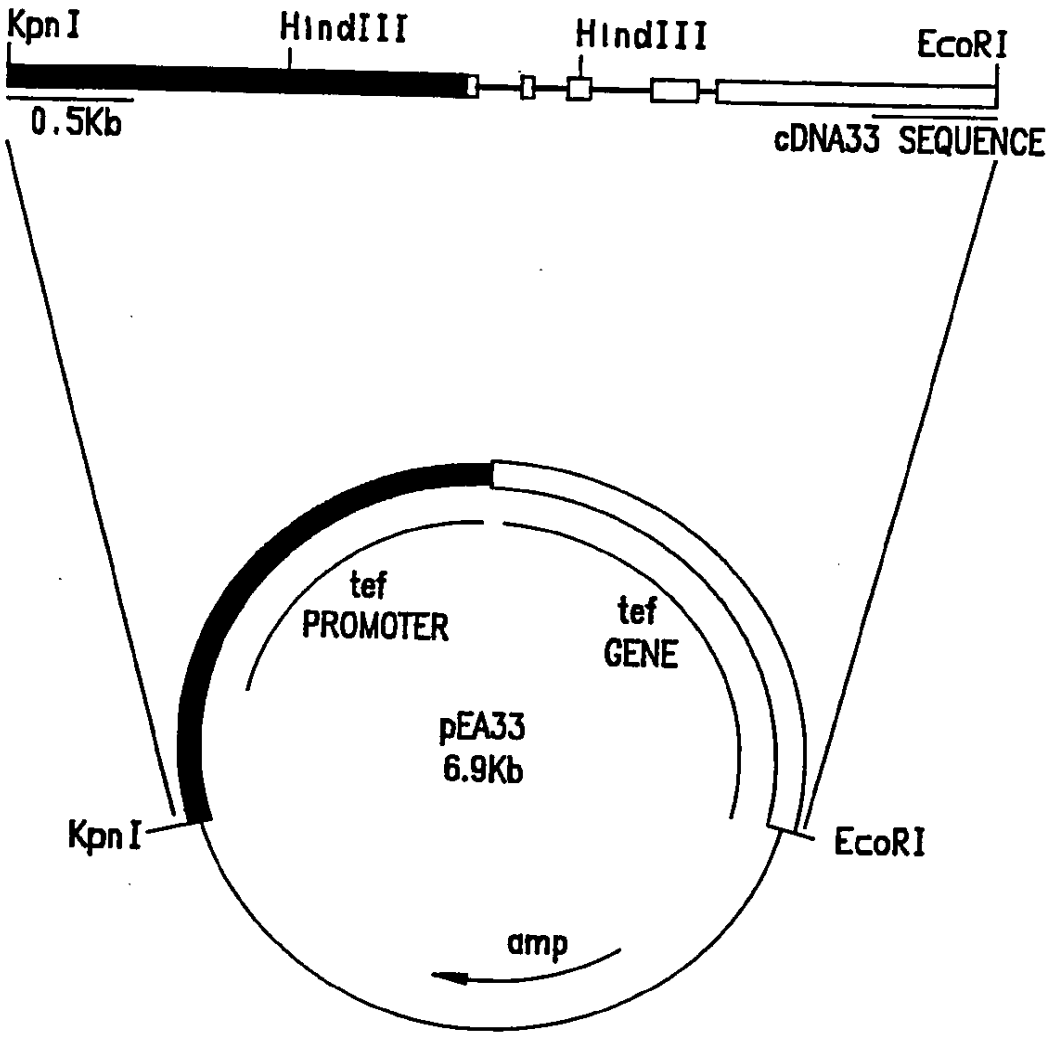
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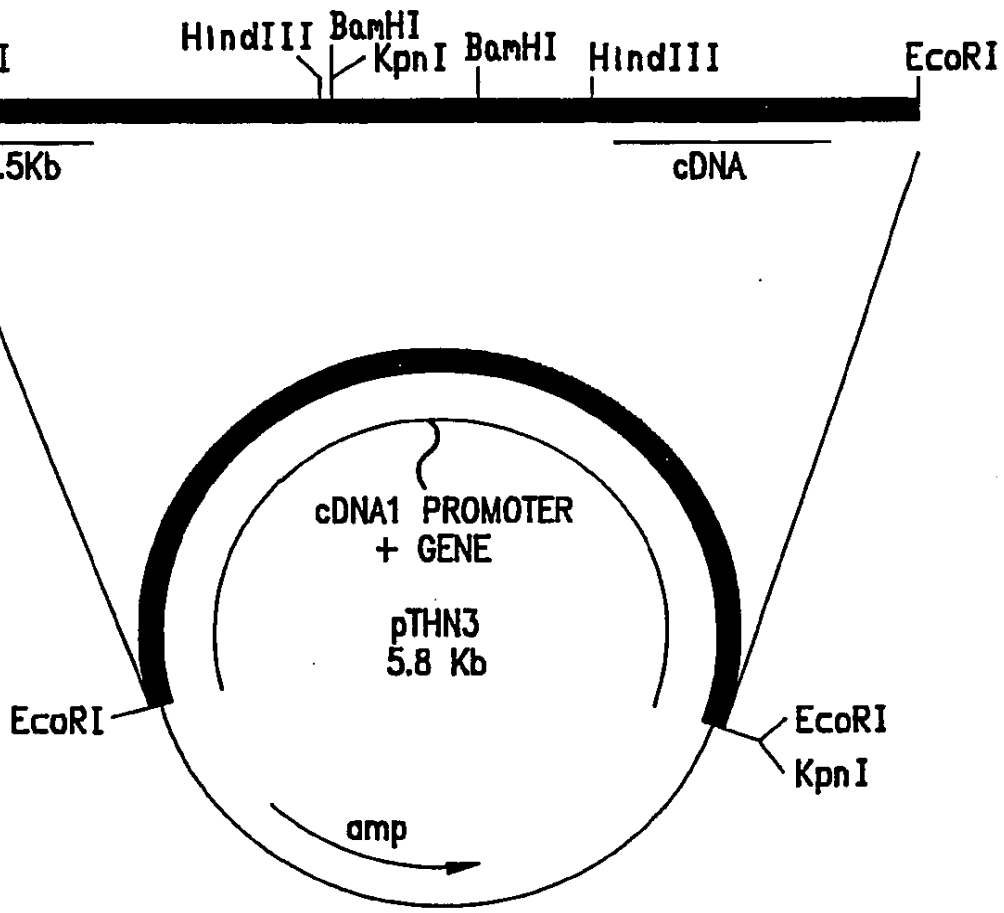
FIG.2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cDNA1 PROMOTER
+ GENE
pTHN3
5.8 Kb
EcoRI
EcoRI
KpnI
amp

FIG.3

```
   1                                                GGTCTGAAGG ACGTGGAATG
  21 ATGGACTTAA TGACAAGAGT TGCCTGGCTA TTGAGCTCTG GTACATGGAT CTCGAACTGA
  81 GAGCGTACAA GTTACATGTA GTAAATCTAG TAGATCTCGC TGAAAGCCCT CTTTCCCGGT
 141 AGAAACACCA CCAGCGTCCC GTAGGACAAG ATCCTGTCGA TCTGAGCACA TGAATTGCTT
 201 CCCTGGATCT GGCGCTGCAT CTGTTTCCCC AGACAATGAT GGTAGCAGCG CATGGAAGAA
 261 CCCGGTTGTT CGGAATGTCC TTGTGCTAAC AGTGGCATGA TTTTACGTTG CGGCTCATCT
 321 CGCCTTGGCA CCGGACCTCA GCAAATCTTG TCACAACAGC AATCTCAAAC AGCCTCATGG
 381 TTCCCAGATT CCCTGATTCA GAACTCTAGA GCGGCAGATG TCAAACGATT CTGACCTAGT
 441 ACCTTGAGCA TCCCTTTCGG ATCCGGCCCA TGTTCTGCCT GCCCTTCTGA GCACAGCAAA
 501 CAGCCCAAAA GGCGCCGGCC GATTCCTTTC CCGGGATGCT CCGGAGTGGC ACCACCTCCC
 561 AAAACAAGCA ACCTTGAACC CCCCCCCCAA ATCAACTGAA GCGCTCTTCG CCTAACCAGC
 621 ATAAGCCCCC CCCAGGATCG TTAGGCCAAG TGGTAGGGCC AGCCAATTAG CGAGNGGCCA
 681 TTTGGAGGTC ATGGGCGCAG AATGTCCTGA CAGTGGTATG ATATTGACTG CCCGGTGTGT
 741 GTGGCATCTG GCCATAATCG CAGGCTGAGG CGAGGAAGTC TCGTGAGGAT GTCCCGACTT
 801 TGACATCATG AGGGAGTGAG AAACTGAAGA GAAGGAAAGC TTCGAAGGTT CGATAAGGGA
 861 TGATTTGCAT GGCGGGCGAC AGGATGCGAT GGCTCGTTGG GATACATAAT GCTTGGGTTG
 921 GAAGCGATTC CAGGTCGTCT TTTTTTGGTT CATCATCACA GCATCAACAA GCAACGATAC
 981 AAGCAATCCA CTGAGGATTA CCTCTCAACT CAACCACTTT CCAAACCATC TCAACTCCCT
1041 AAGATTCTTT CAGTGTATTA TCACTAGGAT TTTTCCCAAG CCGGCTTCAA AACACACAGA
1101 TAAACCACCA ACTCTACAAC CAAAGACTTT TTGATCAATC CAACAACTTC TCTCAACATG
1161 TCTGCTGCAA CCGTCACCCG CACTGCAACC GCCGCTGTTC GCAGACCCGG CTTCTTCATG
1221 CAAGTCCGAC GGATGGGACG CTCATTCGAG CACCAGCCCT TTGAGCGACT CTCCGCCACC
1281 ATGAAGCCTG CACGACCCGA CTATGCTAAG CAAGTCGTCT GGACGGCTGG CAAGTTTGTC
1341 ACTTATGTTC CTCTTTTCGG CGCCATGCTT ACCTGGCCTG CGCTCGCCAA STGGGCTCTG
1401 GACGGACACA TCGGACGGTG GTAAAAGATC AGACTCTTGT CGAGGCAACG GGGAATAGAC
```

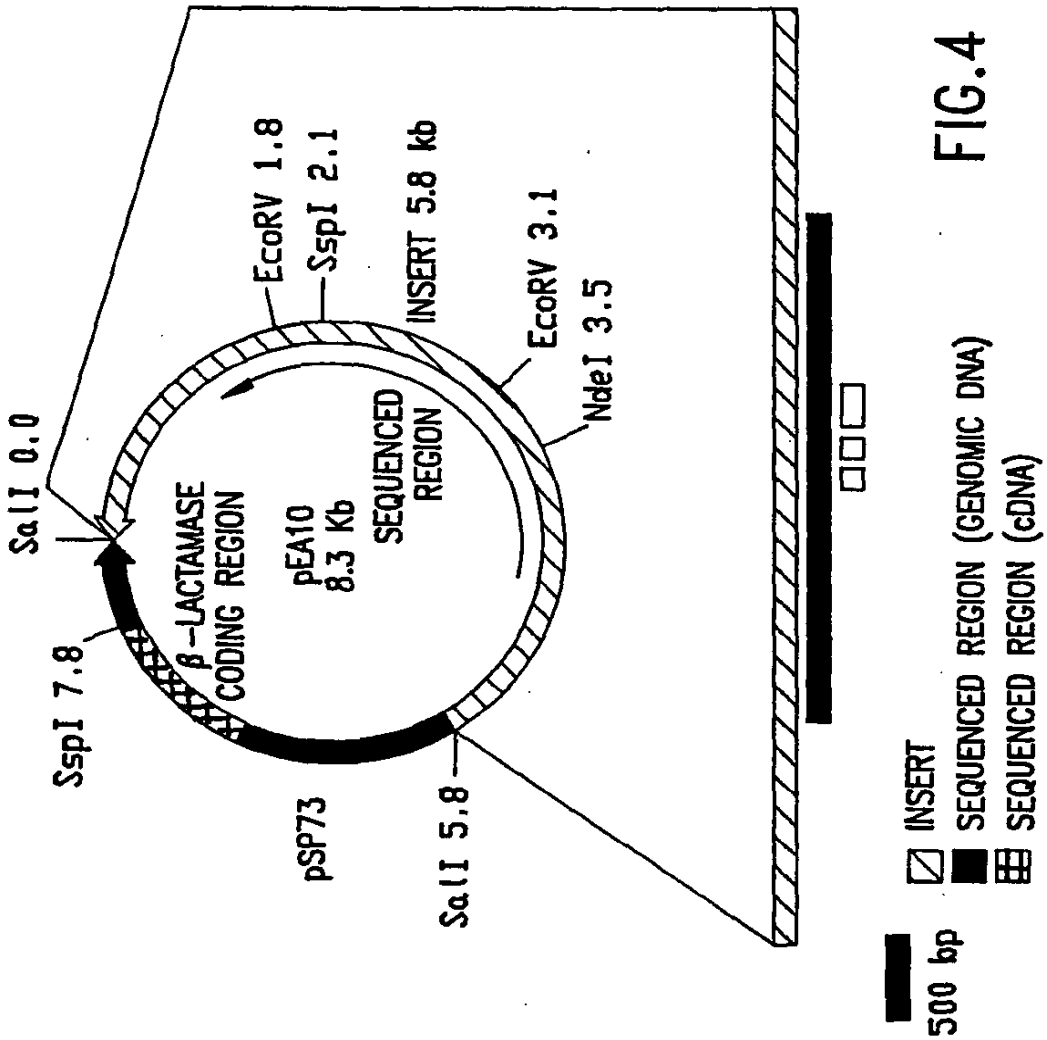
FIG.3A

1461 AGGACAGCAA AAAAGATATC TCCGGATAGA AGTGTCCATC TTTCGACTTG TATATATATA

1521 TATGCTATAC TCTGGGGGCG TTTGGATGGA CTTTGGGCAC GAAGCATACT TTGGCGCAAC

1581 GCAGATACTT TAATCTGATT CCTTTTGTTA ATTCAAAAAA AAAAAAAAAA AAAAAA

FIG.3A(Cont.)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SspI 7.8
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pEA10 8.3 Kb
SEQUENCED REGION
EcoRV 1.8
SspI 2.1
INSERT 5.8 kb
EcoRV 3.1
NdeI 3.5
pSP73
SalI 5.8
500 bp
INSERT
SEQUENCED REGION (GENOMIC DNA)
SEQUENCED REGION (cDNA)

FIG.4

```
          10         20         30         40         50         60
          |          |          |          |          |          |
   1 TTTGTATGGC TGGATCTCGA AAGGCCCTTG TCATCGCCAA GCGTGGCTAA TATCGAATGA
  61 GGGACACCGA CTTGCATATC TCCTGATCAT TCAAACGACA AGTGTGAGGT AGGCAATCCT
 121 CGTATCCCAT TGCTGGGCTG AAAGCTTCAC ACGTATCGCA TAAGCGTCTC CAACCAGTGC
 181 TTAGGTGACC CTTAAGGATA CTTACAGTAA GACTGTATTA AGTCAGTCAC TCTTTCACTC
 241 GGGCTTTGAA TACGATCCTC AATACTCCCG ATAACAGTAA GAGGATGATA CAGCCTGCAG
 301 TTGGCAAATG TAAGCGTAAT TAAACTCAGC TGAACGGCCC TTGTTGAAAG TCTCTCTCGA
 361 TCAAAGCAAA GCTATCCACA GACAAGGGTT AAGCAGGCTC ACTCTTCCTA CGCCTTGGAT
 421 ATGCAGCTTG GCCAGCATCG CGCATGGCCA ATGATGCACC CTTCACGGCC CAACGGATCT
 481 CCCGTTAAAC TCCCCTGTAA CTTGGCATCA CTCATCTGTG ATCCCAACAG ACTGAGTTGG
 541 GGGCTGCGGC TGGCGGATGT CGGAGCAAAG GATCACTTCA AGAGCCCAGA TCCGGTTGGT
 601 CCATTGCCAA TGGATCTAGA TTCGGCACCT TGATCTCGAT CACTGACACA TGGTGAGTTG
 661 CCCGGACGCA CCACAAGTCC CCCTGTGTCA TTGAGTCCCC ATATGCGTCT TCTCAGCGTG
 721 CAACTCTGAG ACGGATTAGT CCTCACGATG AAATTAACTT CCAGCTTAAG TTCGTAGCCT
 781 TGAATGAGTG AAGAAATTTC AAAAACAAAC TGAGTAGAGG TCTTGAGCAG CTGGGGTGGT
 841 ACGCCCCTCC TCGACTCTTG GGACATCGTA CGGCAGAGAA TCAACGGATT_CACACCTTTG
 901 GGTCGAGATG AGCTGATCTC GACAGATACG TGCTTCACCA CAGCTGCAGC TACCTTTGCC
 961 CAACCATTGC GTTCCAGGAT CTTGATCTAC ATCACCGCAG CACCCGAGCC AGGACGGAGA
1021 GAACAATCCG GCCACAGAGC AGCACCGCCT TCCAACTCTG CTCCTGGCAA CGTCACACAA
1081 CCTGATATTA GATATCCACC TGGGTGATTG CCATTGCAGA GAGGTGGCAG TTGGTGATAC
1141 CGACTGGCCA TGCAAGACGC GGCCGGGCTA GCTGAAATGT CCCCGAGAGG ACAATTGGGA
1201 GCGTCTATGA CGGCGTGGAG ACGACGGGAA AGGACTCAGC CGTCATGTTG TGTTGCCAAT
1261 TTGAGATTGT TGACCGGGAA AGGGGGGACG AAGAGGATGG CTGGGTGAGG TGGTATTGGG
1321 AGGATGCATC ATTCGACTCA GTGAGCGATG TAGAGCTCCA AGAATATAAA TATCCCTTCT
1381 CTGTCTTCTC AAAATCTCCT TCCATCTTGT CCTTCATCAG CACCAGAGCC AGCCTGAACA
1441 CCTCCAGTCA ACTTCCCTTA CCAGTACATC TGAATCAACA TCCATTCTTT GAAATCTCAC
1501 CACAACCACC ATCTTCTTCA AAATGAAGTT CTTCGCCATC GCCGCTCTCT TTGCCGCCGC
1561 TGCCGTTGCC CAGCCTCTCG AGGACCGCAG CAACGGCAAC GGCAATGTTT GCCCTCCCGG
1621 CCTCTTCAGC AACCCCCAGT GCTGTGCCAC CCAAGTCCTT GGCCTCATCG GCCTTGACTG
1681 CAAAGTCCGT AAGTTGAGCC ATAACATAAG AATCCTCTTG ACGGAAATAT GCCTTCTCAC
1741 TCCTTTACCC CTGAACAGCC TCCCAGAACG TTTACGACGG CACCGACTTC CGCAACGTCT
1801 GCGCCAAAAC CGGCGCCCAG CCTCTCTGCT GCGTGGCCCC CGTTGTAAGT TGATGCCCCA
1861 GCTCAAGCTC CAGTCTTTGG CAAACCCATT CTGACACCCA GACTGCAGGC CGGCCAGGCT
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FIG.4A

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1921  CTTCTGTGCC AGACCGCCGT CGGTGCTTGA GATGCCCGCC CGGGGTCAAG GTGTGCCCGT
1981  GAGAAAGCCC ACAAAGTGTT GATGAGGACC ATTTCCGGTA CTGGGAAAGT TGGCTCCACG
2041  TGTTTGGGCA GGTTTGGGCA AGTTGTGTAG ATATTCCATT CGTACGCCAT TCTTATTCTC
2101  CAATATTTCA GTACACTTTT CTTCATAAAT CAAAAAGACT GCTATTCTCT TTGTGACATG
2161  CCGGAAGGGA ACAATTGCTC TTGGTCTCTG TTATTTGCAA GTAGGAGTGG GAGATTCGCC
2221  TTAGAGAAAG TAGAGAAGCT GTGCTTGACC GTGGTGTGAC TCGACGAGGA TGGACTGAGA
2281  GTGTTAGGAT TAGGTCGAAC GTTGAAGTGT ATACAGGATC GTCTGGCAAC CCACGGATCC
2341  TATGACTTGA TGCAATGGTG AAGATGAATG ACAGTGTAAG AGGAAAAGGA AATGTCCGCC
2401  TTCAGCTGAT ATCCACGCCA ATGATACAGC GATATACCTC CAATATCTGT GGGAACGAGA
2461  CATGACATAT TTGTGGGAAC AACTTCAAAC AGCGAGCCAA GACCTCAATA TGCACATCCA
2521  AAGCCAAACA TTGGCAAGAC GAGAGACAGT CACATTGTCG TCGAAAGATG GCATCGTACC
2581  CAAATCATCA GCTCTCATTA TCGCCTAAAC CACAGATTGT TTGCCGTCCC CCAACTCCAA
2641  AACGTTACTA CAAAAGACAT GGGCGAATGC AAAGACCTGA AAGCAAACCC TTTTTGCGAC
2701  TCAATTCCCT CCTTTGTCCT CGGAATGATG ATCCTTCACC AAGTAAAAGA AAAAGAAGAT
2761  TGAGATAATA CATGAAAAGC ACAACGGAAA CGAAAGAACC AGGAAAAGAA TAAATCTATC
2821  ACGCACCTTG TCCCCACACT AAAAGCAACA GGGGGGGTAA AATGAAAT
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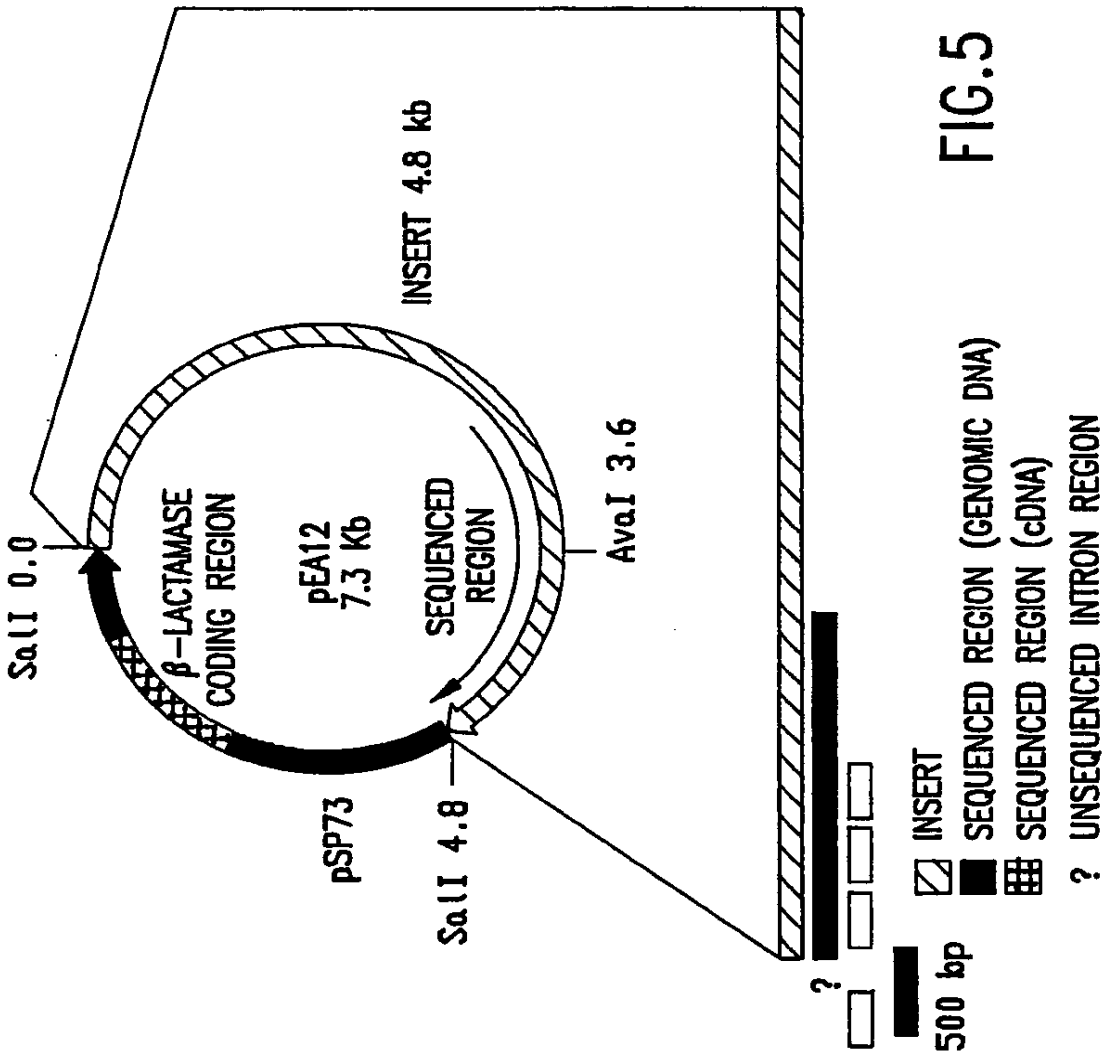
FIG.4A(Cont.)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β-LACTAMASE CODING REGION
pEA12 7.3 Kb
SEQUENCED REGION
AvaI 3.6
pSP73
SalI 4.8
?
500 bp
INSERT
SEQUENCED REGION (GENOMIC DNA)
SEQUENCED REGION (cDNA)
? UNSEQUENCED INTRON REGION

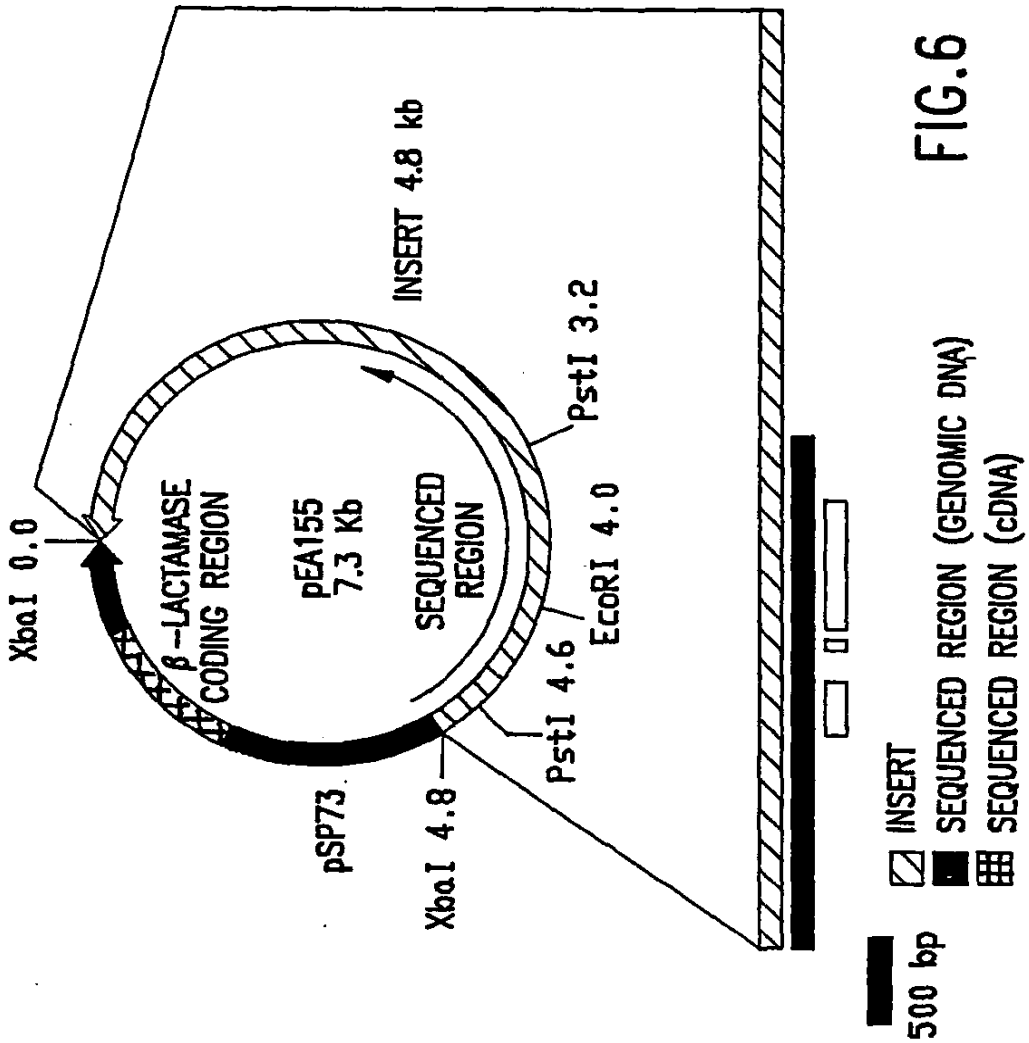
FIG.5

```
                10        20        30        40        50        60
                |         |         |         |         |         |
   1 AAAAAGCTAG AACGAGACGA TTCCGGCCCG GCAAACCAGG CCGAGTGACG GGAGCATTTC
  61 CATGATTTCA CTCGGCAAAC TCTGGCTACA ATTTTCAGGC GGCGAGTTCC GATACAAGGG
 121 AAATCTATTA CCCACAGACG AACGGGAATC GGTGATGAGT GGTTTCTTGT AAGTCAACAT
 181 TGAGCTAGAT AATTCCGGGC GAGATCAAGA TGCCATACTT TGATTGATGA AAAATCAATG
 241 TCAGGCGTAA GTCTCTTCAA GCTCGCCCAG TCCTCTGTAT GTAACAGCAA TCGCAATTCC
 301 GAAATGTGCC GAGCCAATGG AACATGCGTG TCTTTCTCTT TTCACACACA TCCAGTTCGA
 361 GAGTCTTCTC TTCATCGTTT CATCGAATCC CTTCCCCTCC AGCTATTCAC CCAGCCGAGC
 421 CCTTCAGCGC ACCAGCGTAT GTATGTACCC TCGGCTAAGA CGCAACAGAA GCATCATCAA
 481 TATACCTGAT GTACTACTAT CTACTATGAA GCCCAAAAAC CCCTTCGCAG CCCAAATGTA
 541 ACCCAAGCAA CGAATCCCCA ATAAGAGACA ATCCTCAGTG ACCCCCAGAA GAGCACAGAA
 601 TCGAGCTGGT CCTGGTGGGT CGCATTGAGA CCGGTGGAGA TGCGTTCGAT TCGACTGCCG
 661 GAGCTCCCGG GAAGCCGGCA GATGGTCCCA TGCGATGCCC TGCACCGTTT TTGTGAATCG
 721 TCGGCATCGC GAGAAGTGGC CTGCTATGAC GTCGCTTGCA GCTTGGCCGC TCTGTTCGAA
 781 GTTTTTCGAT GTTTTTCTTC ATGCGGGAGA AAGAAAACAT CAGATGACAT GATTATCCGA
 841 ATGGATGGCG GGAGTTATCG TGGTGACGGC TGCTTCATGA GATGAGTATA AATGAGCTTG
 901 TTCGCTCAGC GTGTCATGGA TCTTGTCCAG CTCCAAAGCA TCGGCTTCAG CATCCATCCG
 961 CTTGAACAGA CAGGCACCAG CTTGAATCAG AAGCATACCC TTGATTTGAT ACTCTCTTGG
1021 GAAAAAACAC CACCATCTGT GTAATACTTT GATACCCCCA AAGCTCAAAC GACCGCTTGT
1081 ACATACAATA ACACCGCCAC AATGTTCGCC AACTTGACGC ACGCTACCCT GCGATTCATC
1141 GCCTTCTTCA ACCACCTGAT GATCCTGGCC TCATCAGCCA TCGTCACCGG CCTCGTATCC
1201 TGGTTCCTCG ACAAGTACGA CTACCGCGGC GTGAACATTG TCTACCAGGA AGTCATCGTA
1261 TGTCCTCCCA AGCACCACAT CAAACACACC CCATACCTTG GCTCTCCTCA GCTCCGTCGA
1321 AGCACATAAT ACTAACGCAT GCAACAACTA GGCCACCATA ACTCTGGGCT TCTGGCTCGT
1381 TGGTGCCGTC TTGCCCCTCG TTGGCAGATA CCGCGGCCAC CTGGCCCCTC TCAACCTCAT
```

FIG.5A

1441 CTTCTCCTAC CTCTGGCTCA CCTCTTTCAT CTTCTCCGCG CAGGACTGGA GCAGCGACAA
1501 GTGCAGCTTC GGCCAGCCTG GCGAGGGCCA CTGCAGCCGC AAGAAGGCCA TTGAATCCTT
1561 CAACTTTATC GCATTGTAAG TGCCTACAAG TAATTTGCTA TGTATATGGG AGAGAGAGAG
1621 AAGAAGAAGA ATATGGCTCT AACATGGCAT CTCTACAGCT TCTTCCTCCT CTGCAACACC
1681 CTGGTTGAGA TGCTCCTGCT CCGCGCCGAG TATGCTACCC CCGTTGCTGC TGCTCACAAC
1741 AAGGAGATTT CTGCCGGCCG CCCCTCTGAC AACTCTGTCT AAATAACAAT AGACATGCAT
1801 AGATGAACGG AGACCACTTC TACTTTCTTT GCGAGTTCCT GATCCGTTGA CCTGCAGGTC
1861 GACBBBBBCC GCGCTCGCAT GGTTCATCTG CTACAACAAC ACAATGACAA TCCGAACCAG
1921 TCAATAAACC TCGACAACAC GACGAGTACT TTTGCGGATA GAAAGATACC CATTACACAG
1981 GAGATCAAAT GGGGAAATTG GAAGTGTATG GATGGACGCC CGTGTATAAT GAGGTTGTGA
2041 ACGGGATGGG AGGCAATGAA TAATGGATAA TGAGGTAATG GATAGATTCG GTCGTTTTGA
2101 TACCACAGCT GCACTCTGCT CTACGTCTGT CATTAATGAT ACATACAAAT GATACCTTAT
2161 ACGCTAAAAA AAAAA

FIG.5A(Cont.)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PstI 3.2
EcoRI 4.0
PstI 4.6
XbaI 4.8
pSP73
XbaI 0.0
β-LACTAMASE CODING REGION
pEA155 7.3 Kb
SEQUENCED REGION
INSERT
SEQUENCED REGION (GENOMIC DNA)
SEQUENCED REGION (cDNA)
500 bp

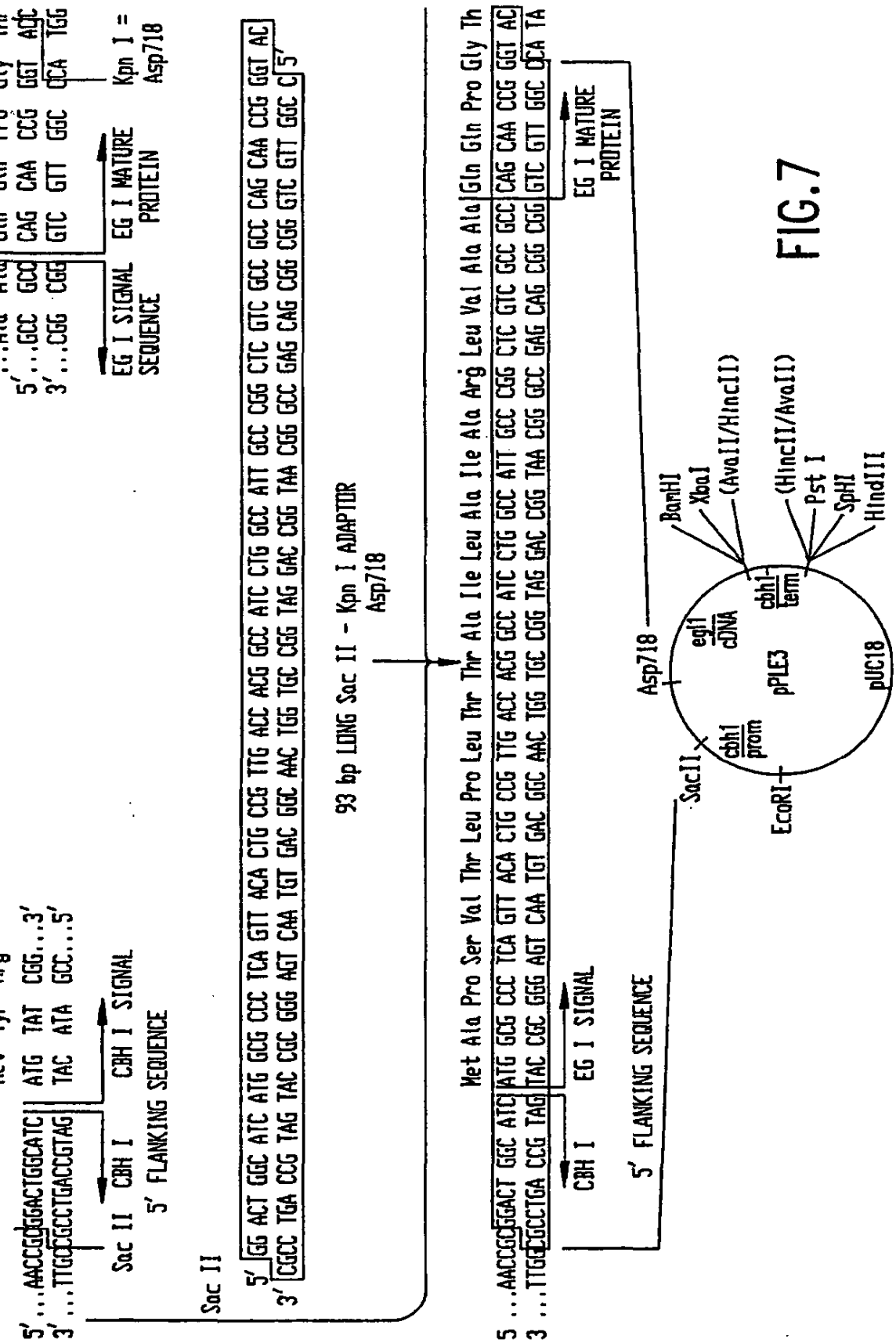
FIG.6

```
                10        20        30        40        50        60
                |         |         |         |         |         |
   1 TCTAGAATCT CTTCGAGATG GCCGAGAAAG GCTTGTTTTT CTCTCCTTCT TCAAACTGGC
  61 CACTGTTTGT TTTCAAACTT GGGGTTTCGT GGGGCTTTTG GGGGCATGTC TGCCAGGTCT
 121 CCCGTAGGCT GGACAGCCAA AGCCTCACTA CAAACAGGCA GTTGTCAATA GATTGATGTC
 181 TGAGATGGAT GGTTTTATGT TTGGGGGAGG TCATGTATGT ATTTATCTAT ATTTGCAAAG
 241 ATGATCCATG AGTCAGACTT GCACAGGTTT CTCGTGCGCT GGATAAATCT TGTTGGAGTG
 301 CGGGTGAGGT GGTGGATGGC ATTCAACCCA CAGCAACACT TGCCCAGGGG GATGTACTGC
 361 AGCGATTTGT TTCCCTTCGA GTATTAGATG ATGATGCCGA ACAGACAAAT TTGAGCCTCG
 421 CTGCTCTCGG ATGTCGGGTT TCTCTTGTGT GCCGGTGATG TGTGATGGCC TGGCCCGCAA
 481 AGAGAGCGAA AAACATGCTC AAAATGTAGC ACACGGCGAC TTCTCGGACA CTTGCGTACC
 541 TTGAGAGACA AGCAGACTAC AGGGATGACG AGTAATACGA CAGAGCGATA CGACACAGCT
 601 ATACGACACA GCTAAGAAAA TAAAGGTATT AGTACTACTA ATTGATTACC TACTACCTAG
 661 ATATATACTA TACCTTATAT TTTATATGTG TGTGTGTGTG TATGTATATG CCTTACCTTA
 721 TGCTTCGCAA AGAAGAGAAA CTAAAACGCC TCCTGGCTAC CTACCTACCT CTACCTTGTA
 781 AGAGATGGAA TAATGTGGCC GCGCGTAAAG TAGGTACTGG ATATACAGGT CCTGAACATG
 841 GCCCTGAATC CTGCCAGGCA GCCACCTCAC CCCTTCCGCA GGTATTTATG TAGCCCACAG
 901 CTCCTCCAGA GACGATGCCG AGATGCCTCA TGCAGTCTAC CTACAAAGCC AGCAGTTTCA
 961 CGCTTGACTC TCACTCTTGA TTGAATTCCC TCCCTCCCAT AATACCAATT GGCGTTCAAC
1021 GATTGCCAGC AGAATGGCCG CCCAACACGA CGTCGAGGCC ATGGCAAAGT CCATGTCCGA
1081 CTTTTTCAAG GACACGGCCC AAAAGCAGGA CTCGACCAAG CATGACTTTG TCCAAGCCTC
1141 GCACGGCATC ATGAGGGCCA TTGTCGAGCC GCTCGTCACC CAGATGGGCT TCCGCGAGAC
1201 CCTCACCGAG CCCGTCGTCT TGCTCGACAG CGCGTGCGGA GCGGGCGTGC TGACGCAGGA
1261 GGTGCAGGCG GCGCTGCCAA AGGAGCTTCT GGAGAGGAGC TCGTTTACGT GTGCGGACAA
1321 TGCCGAGGGC TTGGTGGACG TGGTGAAGAG GAGGATTGAT GAGGAGAAGT GGGTGAATGC
1381 AGAGGCCAAG GTCCTTGATG CCCTGGTGAG TATATACATA TATATCTATA TCTATATAGA
1441 TATATATATG CCTTTGACTC CCCCCTTTAC ATGTCCTACG GCTGCTGATT GATTGATTGA
```

FIG.6A

1501 TGTGGTGATG GTGATGTCCC AGAACACGGG GCTCCCAGAC AACTCCTTCA CCCATGTGGG
1561 CATTGCCCTG GCACTGCACA TCATCCCCGA TCCAGATGCC GTCGTCAAAG GTAAACAATC
1621 ACCAGCGTCA CTGCAAAGAG AGATTACGGG ATATCATATA CTGAAACCAA AGCCCAGACT
1681 GCATCAGAAT GCTCAAGCCA GGCGGCATCT TTGGCGCATC GACATGGCCC AAGGCCAGCG
1741 CCGACATGTT CTGGATCGCC GACATGCGCA CCGCCCTGCA GTCGCTCCCC TTTGACGCGC
1801 CGCTGCCAGA CCCGTTCCCC ATGCAGCTGC ACACCTCGGG CCACTGGGAC GACGCCGCCT
1861 GGGTCGAGAA GCATCTCGTC GAGGATCTGG GGCTGGCCAA CGTCTGTGTG AGGGAGCCGG
1921 CGGGCGAGTA CAGCTTTGCG AGCGCGGACG AGTTCATGGC GACGTTTCAG ATGATGCTGC
1981 CGTGGATTAT GAAGACGTTT TGGAGCGAGG AGGTGAGGGA GAAGCATTCG GTCGACGAGG
2041 TCAAGGAGTT GGTGAAGAGG CATCTGGAGG ACAAGTATGG GGGGAAGGGA TGGACCATTA
2101 AGTGGCGGGT GATTACCATG ACTGCGACTG CGAGCAAGTG AGGGAGGGCA TCTGCTCATG
2161 ATTATGTGAC AGCGAGCCAG TAGAGAGCCA TATTGTTGTC TTCAGAATGT GAGGACCGTG
2221 ATGGTTGGTG TTTGTTGGAG TGATAACTCG TGGGTGTTGC TATTTGCATG TGAGACGATG
2281 AACCATGCGC ACCAGCCACA ATCACTGTCC CCCACCTTAC CTACCAACTT CAAGTTACCA
2341 CCTTACCTTT ACCTGATCTA GCACTGTGGC GCAGCTTGGT TTGACTGCTA GGTACCTACC
2401 TAGTAGTAAT CAGGTACATT CTTCATCCCT GTGTCCTGGT GTCGCAGTTG CAGCTTGTCT
2461 TATCGCTGTG GCCACGCATC GAGTGGCAGC ATCTTCAACT TCAAGTCCCG TCGGTCGCAC
2521 TCTGGCCACG TCGCAGATGG ATCGCAGCGG GATCTGAACC GCTCGCTCGG CAACTGATAC
2581 CAAGTCAACA AACACACGAG ACGACGGGAC GCTGATATAA NNNNGAGGAG GGTAAGAGAA
2641 CTCTACGAGG GGCGGAAACT TGGTCCGACA ATTTCCCTCC CATCTTCACC CTCGACTCGA
2701 ACTCGAACTC GATAGCCGCA CCCTCGACCG ATTGCCC

FIG.6A(Cont.)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5'...GCC GCC CAG CAA CCG GGT ACC
3'...CGG CGG GTC GTT GGC CCA TGG
EG I SIGNAL SEQUENCE
EG I MATURE PROTEIN
Kpn 1 = Asp718
Met Tyr Arg
5'...AACCGCGGACTGGCATC ATG TAT CGG...3'
3'...TTGGCGCCTGACCGTAG TAC ATA GCC...5'
Sac II CBH I CBH I SIGNAL
5' FLANKING SEQUENCE
Sac II
5' GG ACT GGC ATC ATG GCG CCC TCA GTT ACA CTG CCG TTG ACC ACG GCC ATC CTG GCC ATT GCC CGG CTC GTC GCC GCC CAG CAA CCG GGT AC 3'
3' CGCC TGA CCG TAG TAC CGC GGG AGT CAA TGT GAC GGC AAC TGG TGC CGG TAG GAC CGG TAA CGG GCC GAG CAG CGG CGG GTC GTT GGC C 5'
93 bp LONG Sac II - Kpn I ADAPTOR
Asp718
Met Ala Pro Ser Val Thr Leu Pro Leu Thr Thr Ala Ile Leu Ala Ile Ala Arg Leu Val Ala Ala Gln Gln Pro Gly Th
5 ...AACCGCGGACT GGC ATC ATG GCG CCC TCA GTT ACA CTG CCG TTG ACC ACG GCC ATC CTG GCC ATT GCC CGG CTC GTC GCC GCC CAG CAA CCG GGT AC
3 ...TTGGCGCCTGA CCG TAG TAC CGC GGG AGT CAA TGT GAC GGC AAC TGG TGC CGG TAG GAC CGG CAG CAG CGG CGG GTC GTT GGC CCA TA
CBH I EG I SIGNAL
5' FLANKING SEQUENCE
EG I MATURE PROTEIN
Asp718
SacII
EcoRI
cbh1 prom
egl1 cDNA
cbh1 term
pPLE3
pUC18
BamHI
XbaI
(AvaII/HincII)
(HincII/AvaII)
Pst I
SphI
HindIII

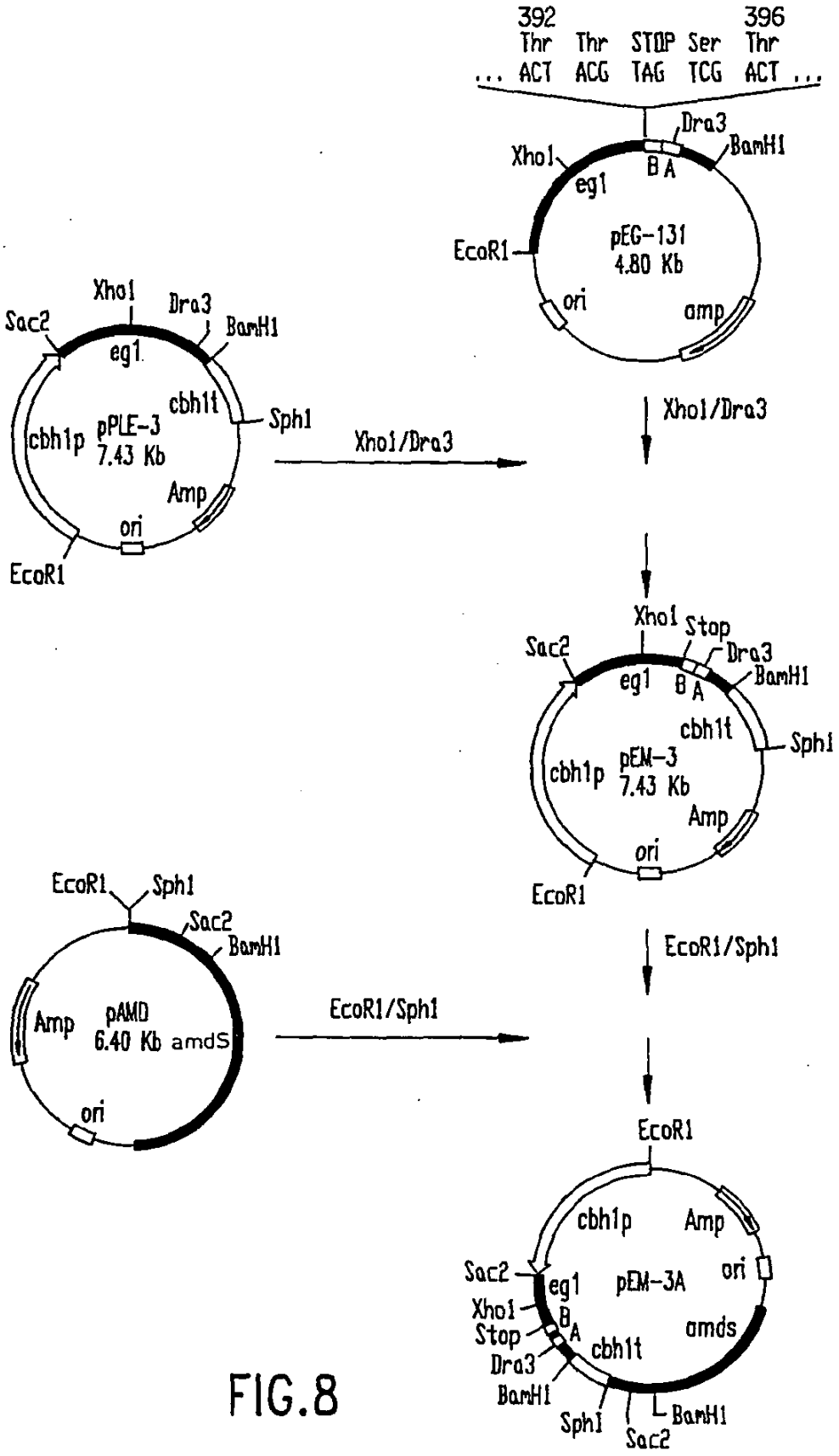
FIG.7

CCCCCCTATC TTAGTCCTTC TTGTTGTCCC AAAATGGCGC CCTCAGTTAC ACTGCCGTTG
ACCACGGCCA TCCTGGCCAT TGCCCGGCTC GTCGCCGCCC AGCAACCGGG TACCAGCACC
CCCGAGGTCC ATCCCAAGTT GACAACCTAC AAGTGTACAA AGTCCGGGGG GTGCGTGGCC
CAGGACACCT CGGTGGTCCT TGACTGGAAC TACCGCTGGA TGCACGACGC AAACTACAAC
TCGTGCACCG TCAACGGCGG CGTCAACACC ACGCTCTGCC CTGACGAGGC GACCTGTGGC
AAGAACTGCT TCATCGAGGG CGTCGACTAC GCCGCCTCGG GCGTCACGAC CTCGGGCAGC
AGCCTCACCA TGAACCAGTA CATGCCCAGC AGCTCTGGCG GCTACAGCAG CGTCTCTCCT
CGGCTGTATC TCCTGGACTC TGACGGTGAG TACGTGATGC TGAAGCTCAA CGGCCAGGAG
CTGAGCTTCG ACGTCGACCT CTCTGCTCTG CCGTGTGGAG AGAACGGCTC GCTCTACCTG
TCTCAGATGG ACGAGAACGG GGGCGCCAAC CAGTATAACA CGGCCGGTGC CAACTACGGG
AGCGGCTACT GCGATGCTCA GTGCCCCGTC CAGACATGGA GGAACGGCAC CCTCAACACT
AGCCACCAGG GCTTCTGCTG CAACGAGATG GATATCCTGG AGGGCAACTC GAGGGCGAAT
GCCTTGACCC CTCACTCTTG CACGGCCACG GCCTGCGACT CTGCCGGTTG CGGCTTCAAC
CCCTATGGCA GCGGCTACAA AAGCTACTAC GGCCCCGGAG ATACCGTTGA CACCTCCAAG
ACCTTCACCA TCATCACCCA GTTCAACACG GACAACGGCT CGCCCTCGGG CAACCTTGTG
AGCATCACCC GCAAGTACCA GCAAAACGGC GTCGACATCC CCAGCGCCCA GCCCGGCGGC
GACACCATCT CGTCCTGCCC GTCCGCCTCA GCCTACGGCG GCCTCGCCAC CATGGGCAAG
GCCCTGAGCA GCGGCATGGT GCTCGTGTTC AGCATTTGGA ACGACAACAG CCAGTACATG
AACTGGCTCG ACAGCGGCAA CGCCGGCCCC TGCAGCAGCA CCGAGGGCAA CCCATCCAAC
ATCCTGGCCA ACAACCCCAA CACGCACGTC GTCTTCTCCA ACATCCGCTG GGGAGACATT
GGGTCTACTA CGAACTCGAC TGCGCCCCCG CCCCCGCCTG CGTCCAGCAC GACGTTTTCG
ACTACACGGA GGAGCTCGAC GACTTCGAGC AGCCCGAGCT GCACGCAGAC TCACTGGGGG
CAGTGCGGTG GCATTGGGTA CAGCGGGTGC AAGACGTGCA CGTCGGGCAC TACGTGCCAG
TATAGCAACG ACTACTACTC GCAATGCCTT TAGAGCGTTG ACTTGCCTCT GGTCTGTCCA
GACGGGGGCA CGATAGAATG CGGGCACGCA GGGAGCTCGT AGACATTGGG CTTAATATAT
AAGACATGCT ATGTTGTATC TACATTAGCA AATGACAAAC AAATGAAAAA GAACTTATCA
AGCAAAAAAA AAAAAAAAAA AAAAAAAA

FIG.7A

GGACCTACCC AGTCTCACTA CGGCCAGTGC GGCGGTATTG GCTACAGCGG CCCCACGGTC
TGCGCCAGCG GCACAACTTG CCAGGTCCTG AACCCTTACT ACTCTCAGTG CCTGTAAAGC
TCCGTGCGAA AGCCTGACGC ACCGGTAGAT TCTTGGTGAG CCCGTATCAT GACGGCGGCG
GGAGCTACAT GGCCCCGGGT GATTTATTTT TTTTGTATCT ACTTCTGACC CTTTTCAAAT
ATACGGTCAA CTCATCTTTC ACTGGAGATG CGGCCTGCTT GGTATTGCGA TGTTGTCAGC
TTGGCAAATT GTGGCTTTCG AAAACACAAA ACGATTCCTT AGTAGCCATG CATTTTAAGA
TAACGGAATA GAAGAAAGAG GAAATTAAAA AAAAAAAAAA AACAAACATC CCGTTCATAA
CCCGTAGAAT CGCCGCTCTT CGTGTATCCC AGTACCACGT CAAAGGTATT CATGATCGTT
CAATGTTGAT ATTGTTCCGC CAGTATGGCT CCACCCCCAT CTCCGCGAAT CTCCTCTTCT
CGAACGCGGT AGTGGCTGCT GCCAATTGGT AATGACCATA GGGAGACAAA CAGCATAATA
GCAACAGTGG AAATTAGTGG CGCAATAATT GAGAACACAG TGAGACCATA GCTGGCGGCC
TGGAAAGCAC TGTTGGAGAC CAACTTGTCC GTTGCGAGGC CAACTTGCAT TGCTGTCAAG
ACGATGACAA CGTAGCCGAG GACCC

FIG.7B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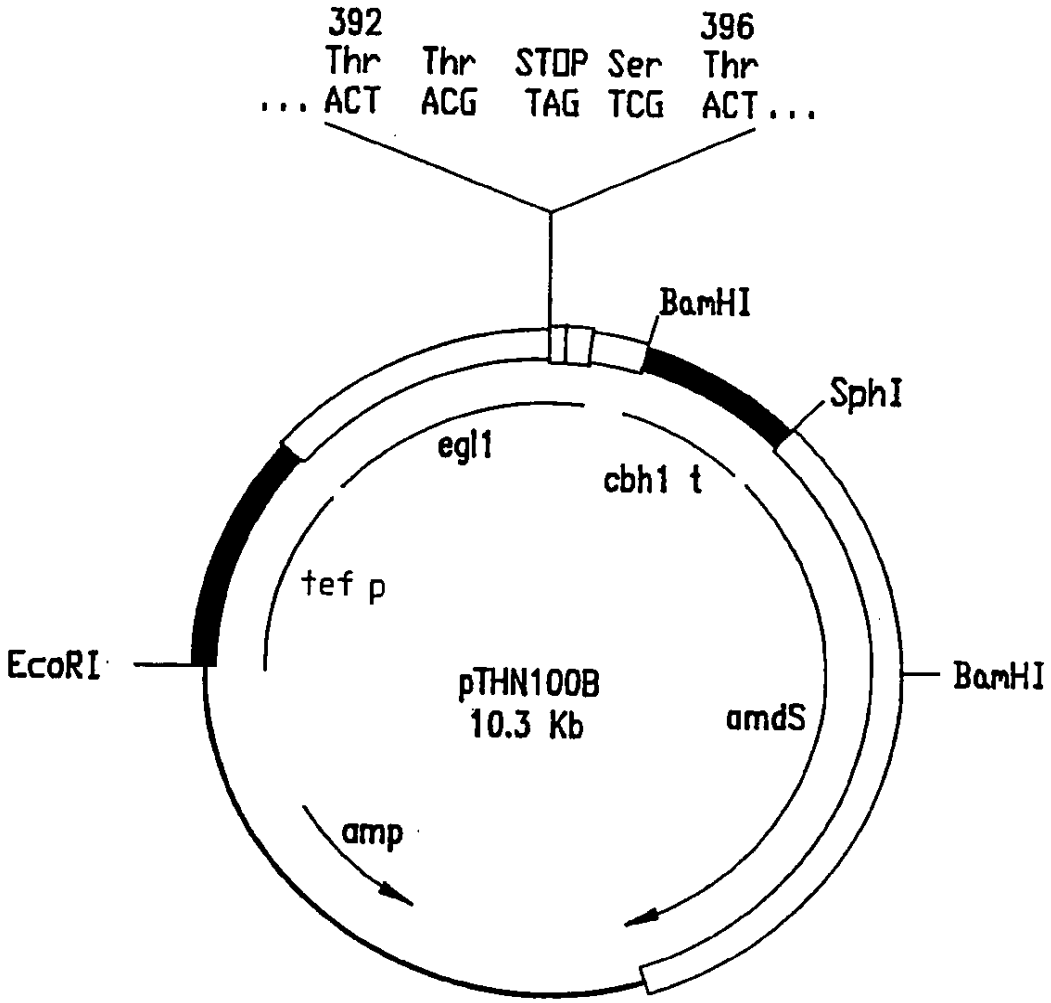
... ACT ACG TAG TCG ACT ...
396
Dra3
BamH1
Xho1
B A
eg1
pEG-131
4.80 Kb
EcoR1
ori
amp
Xho1/Dra3
Xho1
Dra3
Sac2
BamH1
eg1
cbh1t
Sph1
cbh1p
pPLE-3
7.43 Kb
Amp
ori
EcoR1
Xho1/Dra3
Xho1
Stop
Sac2
Dra3
eg1
B A
BamH1
cbh1t
Sph1
cbh1p
pEM-3
7.43 Kb
Amp
ori
EcoR1
EcoR1
Sph1
Sac2
BamH1
Amp
pAMD
6.40 Kb amdS
ori
EcoR1/Sph1
EcoR1/Sph1
EcoR1
cbh1p
Amp
Sac2
eg1
pEM-3A
ori
Xho1
B A
amds
Stop
cbh1t
Dra3
BamH1
Sph1
Sac2
BamH1

FIG.8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Thr
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Thr
ACG
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amdS
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amp

FIG.9

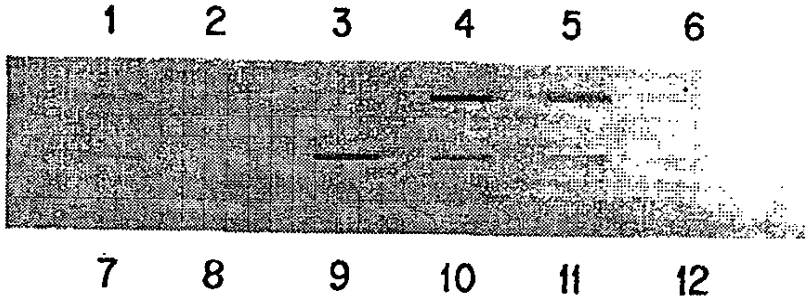

FIG. 10

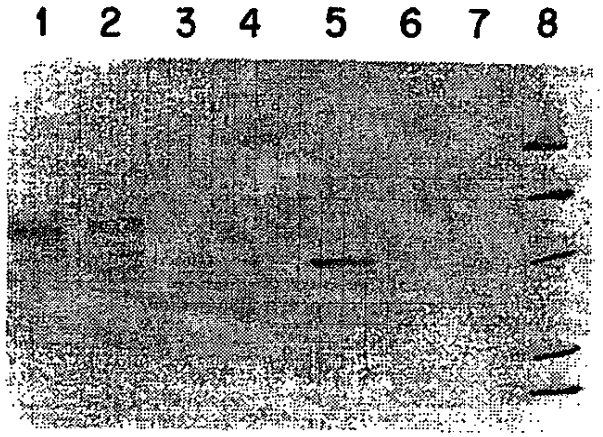

FIG. 11

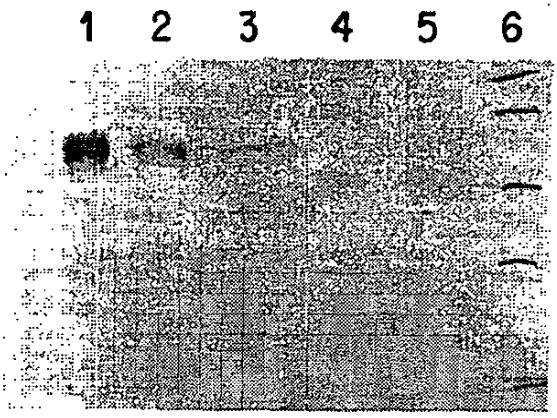

FIG. 12

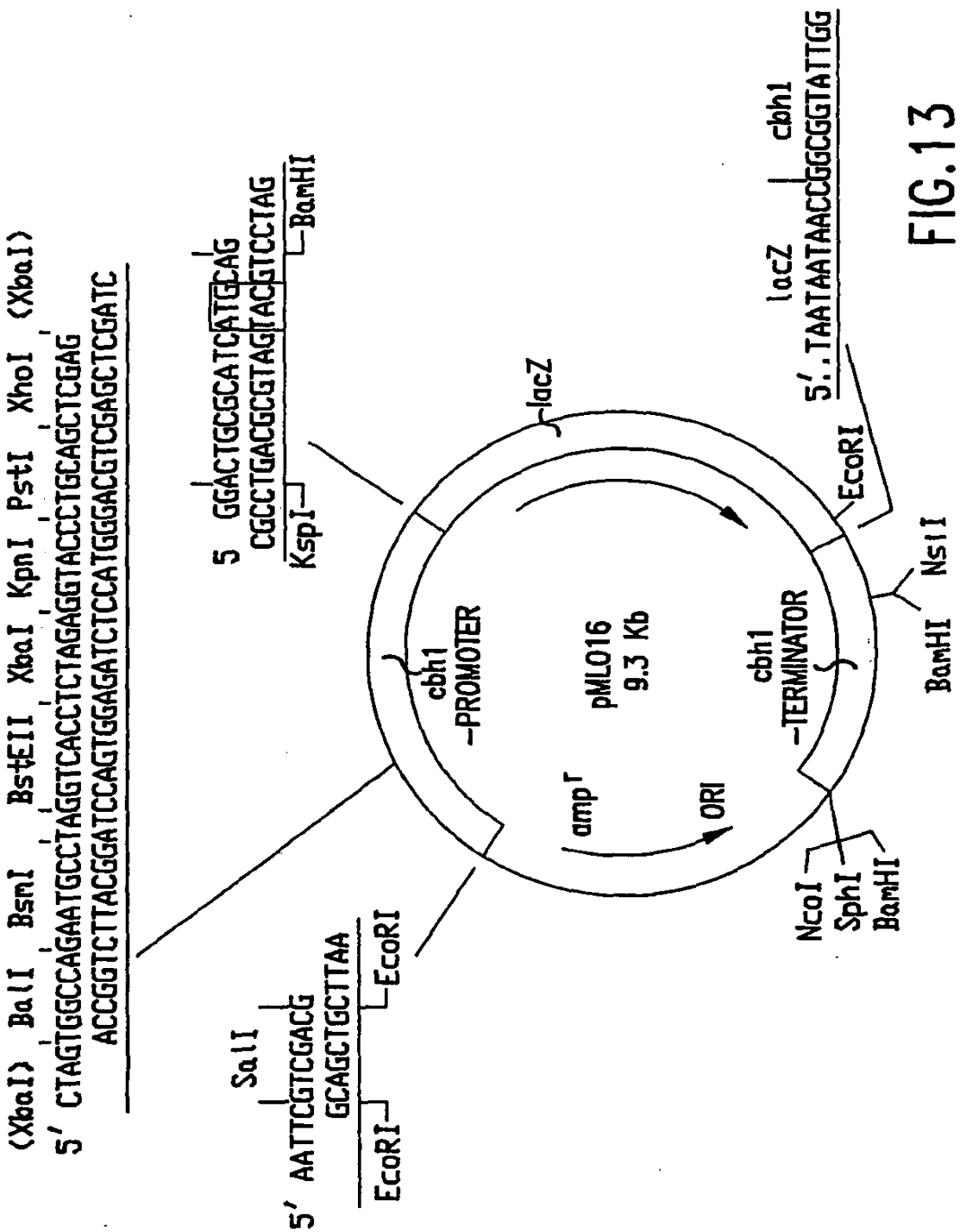
(XbaI) BalI BsmI BstEII XbaI KpnI PstI XhoI (XbaI)
5' CTAGTGGCCAGAATGCCTAGGTCACCTCTAGAGGTACCCTGCAGCTCGAG
ACCGGTCTTACGGATCCAGTGGAGATCTCCATGGGACGTCGAGCTCGATC
5 GGACTGCGCATCATGCAG
CGCCTGACGCGTAGTACGTCCTAG
BamHI
KspI
lacZ
cbh1
-PROMOTER
pML016
9.3 Kb
amp^r
ORI
cbh1
-TERMINATOR
lacZ
cbh1
5'..TAATAATAACCGGCGGTATTGG
EcoRI
BamHI NsiI
NcoI
SphI
BamHI
SalI
5' AATTCGTCGACG
GCAGCTGCTTAA
EcoRI
EcoRI

FIG.13

```
EcoRI  10         20         30         40         50         60
GAATTCTCAC GGTGAATGTA GGCCTTTTGT AGGGTAGGAA TTGTCACTCA AGCACCCCCA      60

ACCTCCATTA CGCCTCCCCC ATAGAGTTCC CAATCAGTGA GTCATGGCAC TGTTCTCAAA     120

TAGATTGGGG AGAAGTTGAC TTCCGCCCAG AGCTGAAGGT CGCACAACCG CATGATATAG     180

GGTCGGCAAC GGCAAAAAAG CACGTGGCTC ACCGAAAAGC AAGATGTTTG CGATCTAACA     240

TCCAGGAACC TGGATACATC CATCATCACG CACGACCACT TTGATCTGCT GGTAAACTCG     300

TATTCGCCCT AAACCGAAGT GCGTGGTAAA TCTACACGTG GGCCCCTTTC GGTATACTGC     360

GTGTGTCTTC TCTAGGTGCA TTCTTTCCTT CCTCTAGTGT TGAATTGTTT GTGTTGGGAG     420

TCCGAGCTGT AACTACCTCT GAATCTCTGG AGAATGGTGG ACTAACGACT ACCGTGCACC     480

TGCATCATGT ATATAATAGT GATCCTGAGA AGGGGGGTTT GGAGCAATGT GGGACTTTGA     540

TGGTCATCAA ACAAAGAACG AAGACGCCTC TTTTGCAAAG TTTTGTTTCG GCTACGGTGA     600

AGAACTGGAT ACTTGTTGTG TCTTCTGTGT ATTTTTGTGG CAACAAGAGG CCAGAGACAA     660
                                    -1505                  XbaI
TCTATTCAAA CACCAAGCTT GCTCTTTTGA GCTACAAGAA CCTGTGGGGT ATATATCTAG     720

AGTTGTGAAG TCGGTAATCC CGCTGTATAG TAATACGAGT CGCATCTAAA TACTCCGAAG     780

CTGCTGCGAA CCCGGAGAAT CGAGATGTGC TGGAAAGCTT CTAGCGAGCG GCTAAATTAG     840

CATGAAAGGC TATGAGAAAT TCTGGAGACG GCTTGTTGAA TCATGGCGTT CCATTCTTCG     900

ACAAGCAAAG CGTTCCGTCG CAGTAGCAGG CACTCATTCC CGAAAAAACT CGGAGATTCC     960

TAAGTAGCGA TGGAACCGGA ATAATATAAT AGGCAATACA TTGAGTTGCC TCGACGGTTG    1020

CAATGCAGGG GTACTGAGCT TGGACATAAC TGTTCCGTAC CCCACCTCTT CTCAACCTTT    1080

GGCGTTTCCC TGATTCAGCG TACCCGTACA AGTCGTAATC ACTATTAACC CAGACTGACC    1140

GGACGTGTTT TGCCCTTCAT TTGGAGAAAT AATGTCATTG CGATGTGTAA TTTGCCTGCT    1200
      -1001
TGACCGACTG GGGCTGTTCG AAGCCCGAAT GTAGGATTGT TATCCGAACT CTGCTCGTAG    1260
```

FIG.13A

```
AGGCATGTTG TGAATCTGTG TCGGGCAGGA CACGCCTCGA AGGTTCACGG CAAGGGAAAC   1320

CACCGATAGC AGTGTCTAGT AGCAACCTGT AAAGCCGCAA TGCAGCATCA CTGGAAAATA   1380

CAAACCAATG GCTAAAAGTA CATAAGTTAA TGCCTAAAGA AGTCATATAC CAGCGGCTAA   1440

                                                    -720
TAATTGTACA ATCAAGTGGC TAAACGTACC GTAATTTGCC AACGCGTTGT GGGGTTGCAG   1500

AAGCAACGGC AAAGCCCACT TCCCACGTTT GTTTCTTCAC TCAGTCCAAT CTCAGCTGGT   1560

GATCCCCCAA TTGGGTCGCT TGTTTGTTCC GGTGAAGTGA AAGAAGACAG AGGTAAGAAT   1620

GTCTGACTCG GAGCGTTTTG CATACAACCA AGGGCAGTGA TGGAAGACAG TGAAATGTTG   1680

ACATTCAAGG AGTATTTAGC CAGGGATGCT TGAGTGTATC GTGTAAGGAG GTTTGTCTGC   1740

CGATACGACG AATACTGTAT AGTCACTTCT GATGAAGTGG TCCATATTGA AATGTAAGTC   1800

GGCACTGAAC AGGCAAAAGA TTGAGTTGAA ACTGCCTAAG ATCTCGGGCC CTCGGGCTTC   1860

GGCTTTGGGT GTACATGTTT GTGCTCCGGG CAAATGCAAA GTGTGGTAGG ATCGACACAC   1920

TGCTGCCTTT ACCAAGCAGC TGAGGGTATG TGATAGGCAA ATGTTCAGGG GCCACTGCAT   1980

GGTTTCGAAT AGAAAGAGAA GCTTAGCCAA GAACAATAGC CGATAAAGAT AGCCTCATTA   2040

AACGAAATGA GCTAGTAGGC AAAGTCAGCG AATGTGTATA TATAAAGGTT CGAGGTCCGT   2100

GCCTCCCTCA TGCTCTCCCC ATCTACTCAT CAACTCAGAT CCTCCAGGAG ACTTGTACAC   2160

CATCTTTTGA GGCACAGAAA CCCAATAGTC AACCGCGGAC TGCGCATCAT G            2211
                                  KspI
```

FIG.13A(Cont.)

```
GGCGGTATTG GCTACAGCGG CCCCACGGTC TGCGCCAGCG GCACAACTTG CCAGGTCCTG      60

AACCCTTACT ACTCTCAGTG CCTGTAAAGC TCCGTGCGAA AGCCTGACGC ACCGGTAGAT     120

TCTTGGTGAG CCCGTATCAT GACGGCGGCG GGAGCTACAT GGCCCCGGGT GATTTATTTT     180

TTTTGTATCT ACTTCTGACC CTTTTCAAAT ATACGGTCAA CTCATCTTTC ACTGGAGATG     240

CGGCCTGCTT GGTATTGCGA TGTTGTCAGC TTGGCAAATT GTGGCTTTCG AAAACACAAA     300

                  NsiI      BamHI
ACGATTCCTT AGTAGCCATG CATCGGGATC CTTTAAGATA ACGGAATAGA AGAAAGAGGA     360

AATTAAAAAA AAAAAAAAAA CAAACATCCC GTTCATAACC CGTAGAATCG CCGCTCTTCG     420

TGTATCCCAG TACCACGGCA AAGGTATTTC ATGATCGTTC AATGTTGATA TTGTTCCCGC     480

CAGTATGGCT GCACCCCCAT CTCCGCGAAT CTCCTCTTCT CGAACGCGGT AGTGGCGCGC     540

CAATTGGTAA TGACCATAGG GAGACAAACA GCATAATAGC AACAGTGGAA ATTAGTGGCG     600

CAATAATTGA GAACACAGTG AGACCATAGC TGGCGGCCTG GAAAGCACTG TTGGAGACCA     660

ACTTGTCCGT TGCGAGGCCA ACTTGCATTG CTGTCAAGAC GATGACAACG TAGCCGAGGA     720

CCGTCACAAG GGACGCAAAG TTGTCGCGGA TGAGGTCTCC GTAGATGGCA TAGCCGGCAA     780

TCCGAGAGTA GCCTCTCAAC AGGTGGCCTT TTCGAAACCG GTAAACCTTG TTCAGACGTC     840

CTAGCCGCAG CTCACCGTAC CAGTATCGAG GATTGACGGC AGAATAGCAG TGGCTCTCCA     900

GGATTTGACT GGACAAAATC TTCCAGTATT CCCAGGTCAC AGTGTCTGGC AGAAGTCCCT     960

TCTCGCGTGC ANTCGAAAGT CGCTATAGTG CGCAATGAGA GCACAGTAGG AGAATAGGAA    1020

CCCGCGAGCA CATTGTTCAA TCTCCACATG AATTGGATGA CTGCTGGGCA GAATGTGCTG    1080

CCTCCAAAAT CCTGCGTCCA ACAGATACTC TGGCAGGGGC TTCAGATGAA TGCCTCTGGG    1140

CCCCCAGATA AGATGCAGCT CTGGATTCTC GGTTACNATG ATATCGCGAG AGAGCACGAG    1200

TTGGTGATGG AGGGACAGGA GGCATAGGTC GCGCAGGCCC ATAACCAGTC TTGCACAGCA    1260

TTGATCTTAC CTCACGAGGA GCTCCTGATG CAGAAACTCC TCCATGTTGC TGATTGGGTT    1320
```

FIG.13B

```
GAGAATTTCA TCGCTCCTGG ATCGTATGGT TGCTGGCAAG ACCCTGCTTA ACCGTGCCGT   1380

GTCATGGTCA TCTCTGGTGG CTTCGTCGCT GGCCTGTCTT TGCAATTCGA CAGCAAATGG   1440

TGGAGATCTC TCTATCGTGA CAGTCATGGT AGCGATAGCT AGGTGTCGTT GCACGCACAT   1500

AGGCCGAAAT GCGAAGTGGA AAGAATTTCC CGGNTGCGGA ATGAAGTCTC GTCATTTTGT   1560

                                          BamHI
ACTCGTACTC GACACCTCCA CCGAAGTGTT AATAATGGAT CCACGATGCC AAAAAGCTTG   1620
 SphI
TGCATGC                                                             1627
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FIG.13B(Cont.)

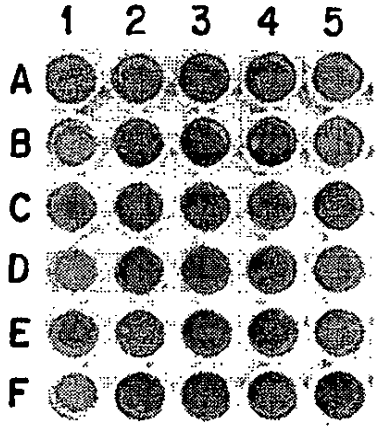
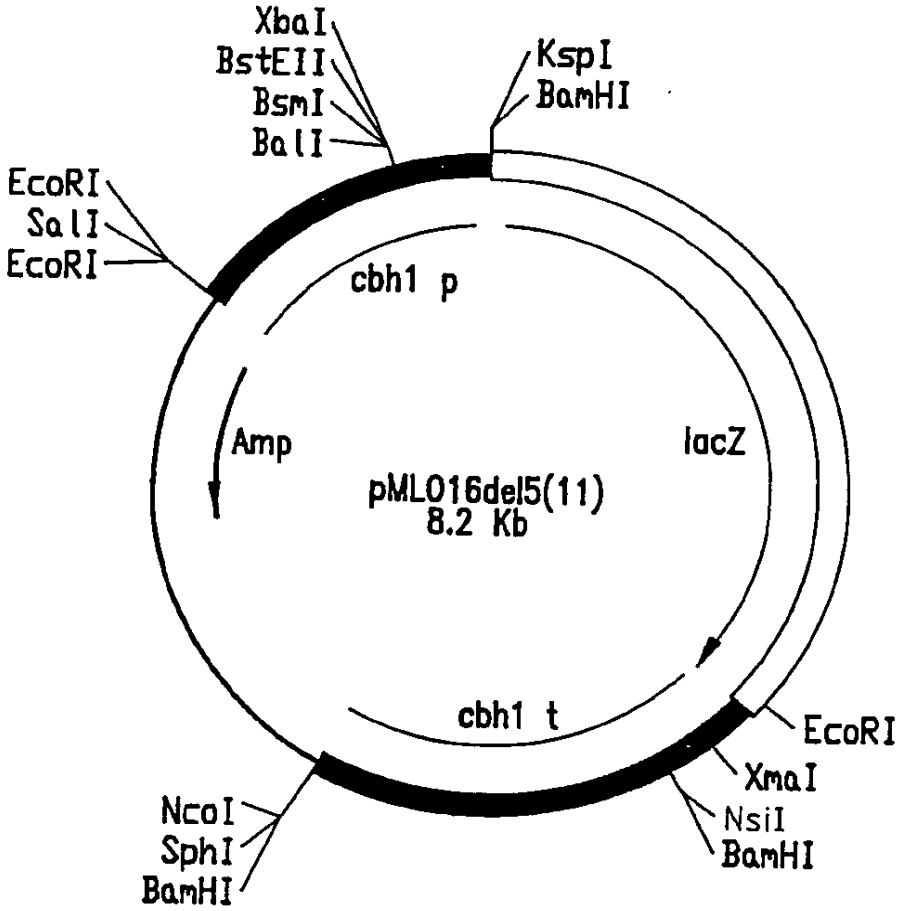


FIG.14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SalI
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lacZ
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8.2 Kb
cbh1 t
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XmaI
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BamHI
NcoI
SphI
BamHI

FIG.15

```
                10        20        30        40        50        60
   1 GAATTCTCAC GGTGAATGTA GGCCTTTTGT AGGGTAGGAA TTGTCACTCA AGCACCCCCA

  61 ACCTCCATTA CGCCTCCCCC ATAGAGTTCC CAATCAGTGA GTCATGGCAC TGTTCTCAAA

 121 TAGATTGGGG AGAAGTTGAC TTCCGCCCAG AGCTGAAGGT CGCACAACCG CATGATATAG

 181 GGTCGGCAAC GGCAAAAAAG CACGTGGCTC ACCGAAAAGC AAGATGTTTG CGATCTAACA

 241 TCCAGGAACC TGGATACATC CATCATCACG CACGACCACT TTGATCTGCT GGTAAACTCG

 301 TATTCGCCCT AAACCGAAGT GCGTGGTAAA TCTACACGTG GGCCCCTTTC GGTATACTGC

 361 GTGTGTCTTC TCTAGGTGCA TTCTTTCCTT CCTCTAGTGT TGAATTGTTT GTGTTGGGAG

 421 TCCGAGCTGT AACTACCTCT GAATCTCTGG AGAATGGTGG ACTAACGACT ACCGTGCACC

 481 TGCATCATGT ATATAATAGT GATCCTGAGA AGGGGGGTTT GGAGCAATGT GGGACTTTGA

 541 TGGTCATCAA ACAAAGAACG AAGACGCCTC TTTTGCAAAG TTTTGTTTCG GCTACGGTGA

 601 AGAACTGGAT ACTTGTTGTG TCTTCTGTGT ATTTTTGTGG CAACAAGAGG CCAGAGACAA

 661 TCTATTCAAA CACCAAGCTT GCTCTTTTGA GCTACAAGAA CCTGTGGGGT ATATATCTAG

 721 TGGCCAGAAT GCCTAGGTCA CCTCTAGAGA GTTGAAACTG CCTAAGATCT CGGGCCCTCG

 781 GGCTTCGGCT TTGGGTGTAC ATGTTTGTGC TCCGGGCAAA TGCAAAGTGT GGTAGGATCG

 841 ACACACTGCT GCCTTTACCA AGCAGCTGAG GGTATGTGAT AGGCAAATGT TCAGGGGCCA

 901 CTGCATGGTT TCGAATAGAA AGAGAAGCTT AGCCAAGAAC AATAGCCGAT AAAGATAGCC

 961 TCATTAAACG AAATGAGCTA GTAGGCAAAG TCAGCGAATG TGTATATATA AAGGTTCGAG

1021 GTCCGTGCCT CCCTCATGCT CTCCCCATCT ACTCATCAAC TCAGATCCTC CAGGAGACTT

1081 GTACACCATC TTTTGAGGCA CAGAAACCCA ATAGTCAACC GCGGACTGCG CATCATG
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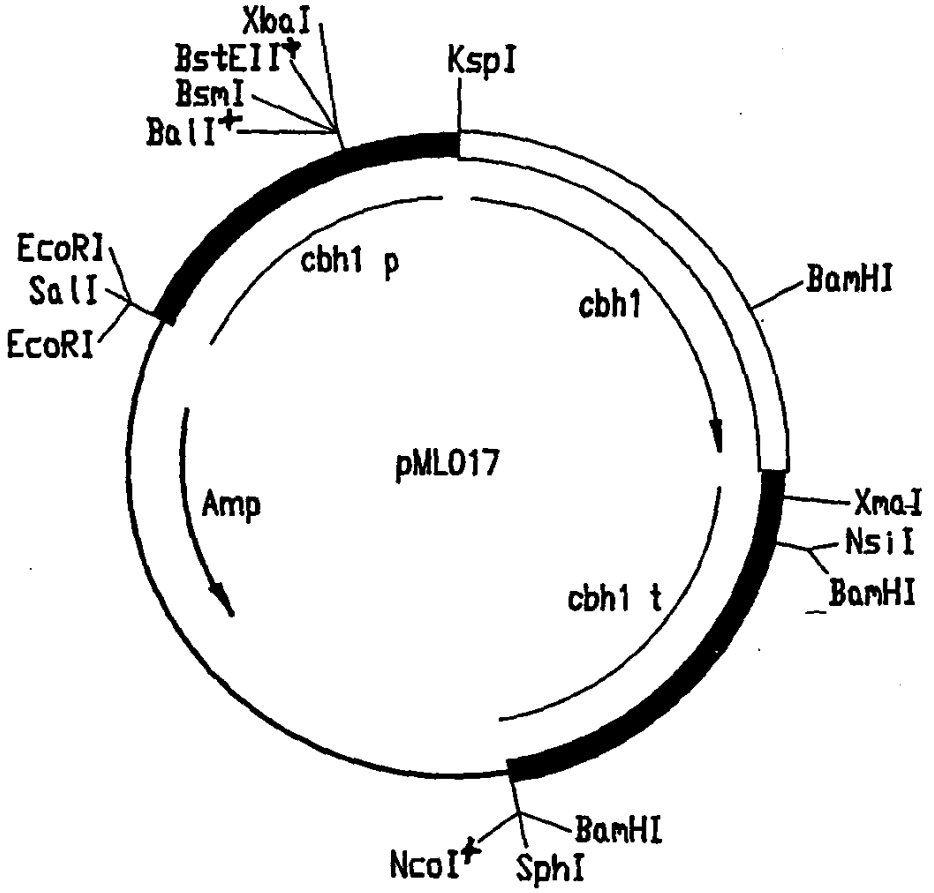
FIG. 15A



- RESTRICTION SITES MARKED WITH + ARE NOT SINGLE SITES
- TWO ADDITIONAL EcoRI -SITES IN THE cbh1-GENE

FIG.16

KspI
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ATCGGAAGTT GGCCGTCATC TCGGCCTTCT TGGCCACAGC TCGTGCTCAG TCGGCCTGCA 1800

CTCTCCAATC GGAGACTCAC CCGCCTCTGA CATGGCAGAA ATGCTCGTCT GGTGGCACTT 1860

GCACTCAACA GACAGGCTCC GTGGTCATCG ACGCCAACTG GCGCTGGACT CACGCTACGA 1920

ACAGCAGCAC GAACTGCTAC GATGGCAACA CTTGGAGCTC GACCCTATGT CCTGACAACG 1980

AGACCTGCGC GAAGAACTGC TGTCTGGACG GTGCCGCCTA CGCGTCCACG TACGGAGTTA 2040

CCACGAGCGG TAACAGCCTC TCCATTGGCT TTGTCACCCA GTCTGCGCAG AAGAACGTTG 2100

GCGCTCGCCT TTACCTTATG GGCAGCGACA CGACCTACCA GGAATTCACC CTGCTTGGCA 2160

ACGAGTTCTC TTTCGATGTT GATGTTTCGC AGCTGCCGTA AGTGACTTAC CATGAACCCC 2220

TGACGTATCT TCTTGTGGGC TCCCAGCTGA CTGGCCAATT TAAGGTGCGG CTTGAACGGA 2280

GCTCTCTACT TCGTGTCCAT GGACGCGGAT GGTGGCGTGA GCAAGTATCC CACCAACACC 2340

GCTGGCGCCA AGTACGGCAC GGGGTACTGT GACAGCCAGT GTCCCCGCGA TCTGAAGTTC 2400

ATCAATGGCC AGGCCAACGT TGAGGGCTGG GAGCCGTCAT CCAACAACGC AAACACGGGC 2460

ATTGGAGGAC ACGGAAGCTG CTGCTCTGAG ATGGATATCT GGGAGGCCAA CTCCATCTCC 2520

GAGGCTCTTA CCCCCCACCC TTGCACGACT GTCGGCCAGG AGATCTGCGA GGGTGATGGG 2580

TGCGGCGGAA CTTACTCCGA TAACAGATAT GGCGGCACTT GCGATCCCGA TGGCTGCGAC 2640

TGGAACCCAT ACCGCCTGGG CAACACCAGC TTCTACGGCC CTGGCTCAAG CTTTACCCTC 2700

GATACCACCA AGAAATTGAC CGTTGTCACC CAGTCCGAGA CGTCGGGTGC CATCAACCGA 2760

TACTATGTCC AGAATGGCGT CACTTTCCAG CAGCCCAACG CCGAGCTTGG TAGTTACTCT 2820

GGCAACGAGC TCAACGATGA TTACTGCACA GCTGAGGAGG CAGAATTCGG CGGATCCTCT 2880

TTCTCAGACA AGGGCGGCCT GACTCAGTTC AAGAAGGCTA CCTCTGGCGG CATGGTTCTG 2940

GTCATGAGTC TGTGGGATGA TGTGAGTTTG ATGGACAAAC ATGCGCGTTG ACAAAGAGTC 3000

FIG.16A

AAGCAGCTGA CTGAGATGTT ACAGTACTAC GCCAACATGC TGTGGCTGGA CTCCACCTAC 3060

CCGACAAACG AGACCTCCTC CACACCCGGT GCCGTGCGCG GAAGCTGCTC CACCAGCTCC 3120

GGTGTCCCTG CTCAGGTCGA ATCTCAGTCT CCCAACGCCA AGGTCACCTT CTCCAACATC 3180

AAGTTCGGAC CCATTGGCAG CACCGGCAAC CCTAGCGGCG GCAACCCTCC CGGCGGAAAC 3240

CCGCCTGGCA CCACCACCAC CCGCCGCCCA GCCACTACCA CTGGAAGCTC TCCCGGACCT 3300

ACCCAGTCTC ACTACGGCCA GTGCGGCGGT ATTGGCTACA GCGGCCCCAC GGTCTGCGCC 3360

AGCGGCACAA CTTGCCAGGT CCTGAACCCT TACTACTCTC AGTGCCTGTA AAGCTCCGTG 3420

CGAAAGCCTG ACGCACCGGT AGATTCTTGG TGAGCCCGTA TCATGACGGC GGCGGGAGCT 3480

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XmaI

FIG.16A(Cont.)

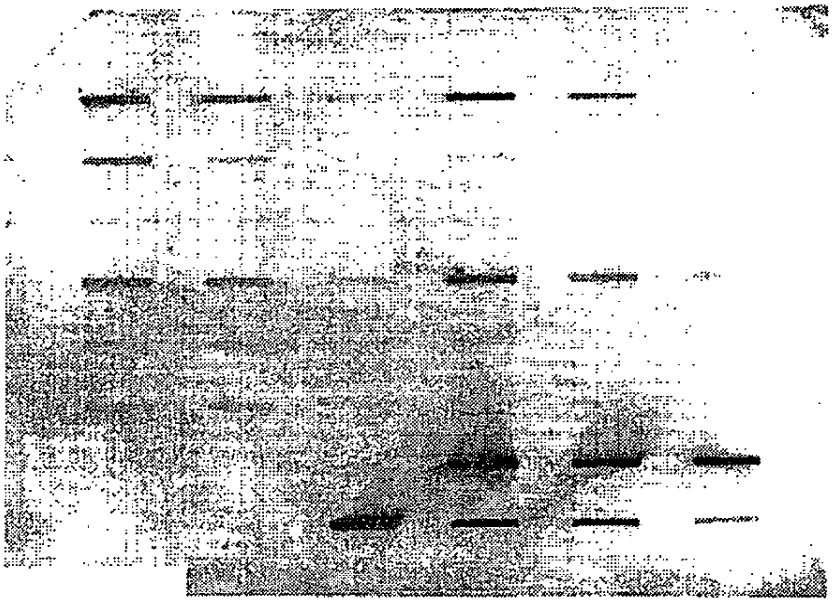
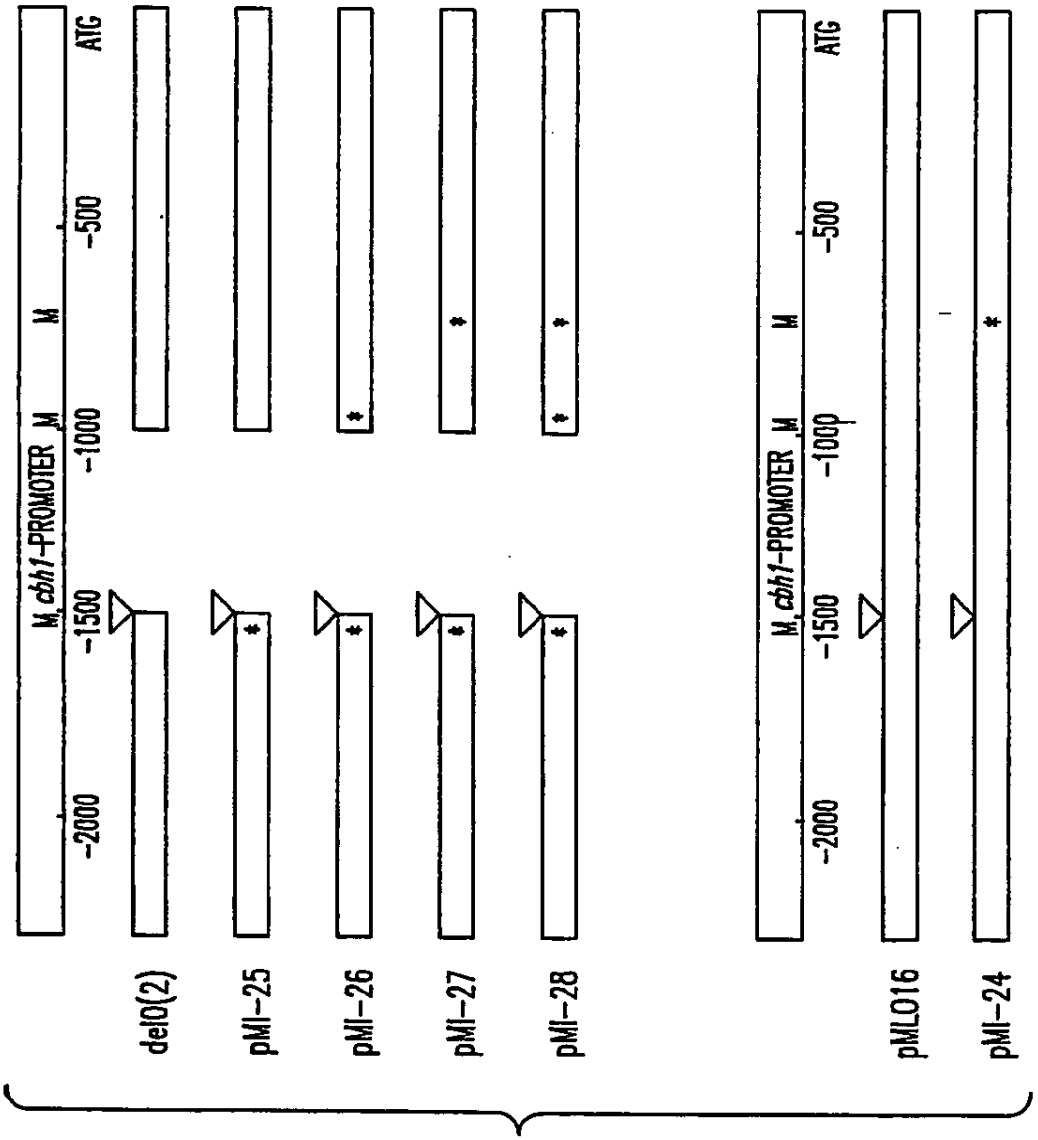


FIG.17A

41A UNDILUTED	41A 1:5	41A 1:50	41B UNDILUTED	41B 1:5	41B 1:50
41E UNDILUTED	41E 1:5	41E 1:50	35A UNDILUTED	35A 1:5	35A 1:50
35B UNDILUTED	35B 1:5	35B 1:50	35C UNDILUTED	35C 1:5	35C 1:50
24A UNDILUTED	24A 1:5	24A 1:50	24B UNDILUTED	24B 1:5	24B 1:50
39A UNDILUTED	39A 1:5	39A 1:50	39B UNDILUTED	39B 1:5	39B 1:50
39C UNDILUTED	39C 1:5	39C 1:50	32D UNDILUTED	32D 1:5	32D 1:50
CBHI NEGATIVE STRAIN UNDILUTED	HOST STRAIN UNDILUTED	BUFFER	HOST STRAIN CELLULOSE MEDIUM 1:20	HOST STRAIN CELLULOSE MEDIUM 1:40	HOST STRAIN CELLULOSE MEDIUM 1:80
CBHI NEGATIVE STRAIN 1:5	HOST STRAIN 1:5	CBHI PROTEIN 200 ng	CBHI PROTEIN 100 ng	CBHI PROTEIN 50 ng	CBHI PROTEIN 25 ng

FIG.17B

FIG.18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pMI-24

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                |         |         |         |         |         |
   1 GAATTCTCAC GGTGAATGTA GGCCTTTTGT AGGGTAGGAA TTGTCACTCA AGCACCCCCA
  61 ACCTCCATTA CGCCTCCCCC ATAGAGTTCC CAATCAGTGA GTCATGGCAC TGTTCTCAAA
 121 TAGATTGGGG AGAAGTTGAC TTCCGCCCAG AGCTGAAGGT CGCACAACCG CATGATATAG
 181 GGTCGGCAAC GGCAAAAAAG CACGTGGCTC ACCGAAAAGC AAGATGTTTG CGATCTAACA
 241 TCCAGGAACC TGGATACATC CATCATCACG CACGACCACT TTGATCTGCT GGTAAACTCG
 301 TATTCGCCCT AAACCGAAGT GCGTGGTAAA TCTACACGTG GGCCCCTTTC GGTATACTGC
 361 GTGTGTCTTC TCTAGGTGCA TTCTTTCCTT CCTCTAGTGT TGAATTGTTT GTGTTGGGAG
 421 TCCGAGCTGT AACTACCTCT GAATCTCTGG AGAATGGTGG ACTAACGACT ACCGTGCACC
 481 TGCATCATGT ATATAATAGT GATCCTGAGA AGGGGGGTTT GGAGCAATGT GGGACTTTGA
 541 TGGTCATCAA ACAAAGAACG AAGACGCCTC TTTTGCAAAG TTTTGTTTCG GCTACGGTGA
 601 AGAACTGGAT ACTTGTTGTG TCTTCTGTGT ATTTTTGTGG CAACAAGAGG CCAGAGACAA
 661 TCTATTCAAA CACCAAGCTT GCTCTTTTGA GCTACAAGAA CCTGTGGGGT ATATATCTAG
 721 TGGCCAGAAT GCCTAGGTCA CCTCTAAAGG TACCCTGCAG CTCGAGCTAG AGTTGTGAAG
 781 TCGGTAATCC CGCTGTATAG TAATACGAGT CGCATCTAAA TACTCCGAAG CTGCTGCGAA
 841 CCCGGAGAAT CGAGATGTGC TGGAAAGCTT CTAGCGAGCG GCTAAATTAG CATGAAAGGC
 901 TATGAGAAAT TCTGGAGACG GCTTGTTGAA TCATGGCGTT CCATTCTTCG ACAAGCAAAG
 961 CGTTCCGTCG CAGTAGCAGG CACTCATTCC CGAAAAAACT CGGAGATTCC TAAGTAGCGA
1021 TGGAACCGGA ATAATATAAT AGGCAATACA TTGAGTTGCC TCGACGGTTG CAATGCAGGG
1081 GTACTGAGCT TGGACATAAC TGTTCCGTAC CCCACCTCTT CTCAACCTTT GGCGTTTCCC
1141 TGATTCAGCG TACCCGTACA AGTCGTAATC ACTATTAACC CAGACTGACC GGACGTGTTT
1201 TGCCCTTCAT TTGGAGAAAT AATGTCATTG CGATGTGTAA TTTGCCTGCT TGACCGACTG
1261 GGGCTGTTCG AAGCCCGAAT GTAGGATTGT TATCCGAACT CTGCTCGTAG AGGCATGTTG
1321 TGAATCTGTG TCGGGCAGGA CACGCCTCGA AGGTTCACGG CAAGGGAAAC CACCGATAGC
1381 AGTGTCTAGT AGCAACCTGT AAAGCCGCAA TGCAGCATCA CTGGAAAATA CAAACCAATG
1441 GCTAAAAGTA CATAAGTTAA TGCCTAAAGA AGTCATATAC CAGCGGCTAA TAATTGTACA
1501 ATCAAGTGGC TAAACGTACC GTAATTTGCC AACGCGTTTC TAGATTGCAG AAGCACGGCA
```

FIG.18A

1561 AAGCCCACTT ACCCACGTTT GTTTCTTCAC TCAGTCCAAT CTCAGCTGGT GATCCCCCAA
1621 TTGGGTCGCT TGTTTGTTCC GGTGAAGTGA AAGAAGACAG AGGTAAGAAT GTCTGACTCG
1681 GAGCGTTTTG CATACAACCA AGGGCAGTGA TGGAAGACAG TGAAATGTTG ACATTCAAGG
1741 AGTATTTAGC CAGGGATGCT TGAGTGTATC GTGTAAGGAG GTTTGTCTGC CGATACGACG
1801 AATACTGTAT AGTCACTTCT GATGAAGTGG TCCATATTGA AATGTAAGTC GGCACTGAAC
1861 AGGCAAAAGA TTGAGTTGAA ACTGCCTAAG ATCTCGGGCC CTCGGGCTTC GGCTTTGGGT
1921 GTACATGTTT GTGCTCCGGG CAAATGCAAA GTGTGGTAGG ATCGACACAC TGCTGCCTTT
1981 ACCAAGCAGC TGAGGGTATG TGATAGGCAA ATGTTCAGGG GCCACTGCAT GGTTTCGAAT
2041 AGAAAGAGAA GCTTAGCCAA GAACAATAGC CGATAAAGAT AGCCTCATTA AACGAAATGA
2101 GCTAGTAGGC AAAGTCAGCG AATGTGTATA TATAAAGGTT CGAGGTCCGT GCCTCCCTCA
2161 TGCTCTCCCC ATCTACTCAT CAACTCAGAT CCTCCAGGAG ACTTGTACAC CATCTTTTGA
2221 GGCACAGAAA CCCAATAGTCAACCGCGGAC TGCGCATCAT G

FIG.18A(Cont.)

```
                10        20        30        40        50        60
                |         |         |         |         |         |
   1 CAATTCTCAC GGTGAATGTA GGCCTTTTGT AGGGTAGGAA TTGTCACTCA AGCACCCCCA
  61 ACCTCCATTA CGCCTCCCCC ATAGAGTTCC CAATCAGTGA GTCATGGCAC TGTTCTCAAA
 121 TAGATTGGGG AGAAGTTGAC TTCCGCCCAG AGCTGAAGGT CGCACAACCG CATGATATAG
 181 GGTCGGCAAC GGCAAAAAAG CACGTGGCTC ACCGAAAAGC AAGATGTTTG CGATCTAACA
 241 TCCAGGAACC TGGATACATC CATCATCACG CACGACCACT TTGATCTGCT GGTAAACTCG
 301 TATTCGCCCT AAACCGAAGT GCGTGGTAAA TCTACACGTG GGCCCCTTTC GGTATACTGC
 361 GTGTGTCTTC TCTAGGTGCA TTCTTTCCTT CCTCTAGTGT TGAATTGTTT GTGTTGGGAG
 421 TCCGAGCTGT AACTACCTCT GAATCTCTGG AGAATGGTGG ACTAACGACT ACCGTGCACC
 481 TGCATCATGT ATATAATAGT GATCCTGAGA AGGGGGGTTT GGAGCAATGT GGGACTTTGA
 541 TGGTCATCAA ACAAAGAACG AAGACGCCTC TTTTGCAAAG TTTTGTTTCG GCTACGGTGA
 601 AGAACTGGAT ACTTGTTGTG TCTTCTGTGT ATTTTTGTGG CAACAAGAGG CCAGAGACAA
 661 TCTATTCAAA CACCAAGCTT GCTCTTTTGA GCTACAAGAA CCTTCTAAAT ATATATCTAG
 721 TGGCCAGAAT GCCTAGGTCA CCTCTAAATG TGTAATTTGC CTGCTTGACC GATCTAAACT
 781 GTTCGAAGCC CGAATGTAGG ATTGTTATCC GAACTCTGCT CGTAGAGGCA TGTTGTGAAT
 841 CTGTGTCGGG CAGGACACGC CTCGAAGGTT CACGGCAAGG GAAACCACCG ATAGCAGTGT
 901 CTAGTAGCAA CCTGTAAAGC CGCAATGCAG CATCACTGGA AAATACAAAC CAATGGCTAA
 961 AAGTACATAA GTTAATGCCT AAAGAAGTCA TATACCAGCG GCTAATAATT GTACAATCAA
1021 GTGGCTAAAC GTACCGTAAT TTGCCAACGC GTTTCTAGAT TGCAGAAGCA CGGCAAAGCC
1081 CACTTACCCA CGTTTGTTTC TTCACTCAGT CCAATCTCAG CTGGTGATCC CCCAATTGGG
1141 TCGCTTGTTT GTTCCGGTGA AGTGAAAGAA GACAGAGGTA AGAATGTCTG ACTCGGAGCG
1201 TTTTGCATAC AACCAAGGGC AGTGATGGAA GACAGTGAAA TGTTGACATT CAAGGAGTAT
1261 TTAGCCAGGG ATGCTTGAGT GTATCGTGTA AGGAGGTTTG TCTGCCGATA CGACGAATAC
```

FIG.18B

1321 TGTATAGTCA CTTCTGATGA AGTGGTCCAT ATTGAAATGT AAGTCGGCAC TGAACAGGCA
1381 AAAGATTGAG TTGAAACTGC CTAAGATCTC GGGCCCTCGG GCTTCGGCTT TGGGTGTACA
1441 TGTTTGTGCT CCGGGCAAAT GCAAAGTGTG GTAGGATCGA CACACTGCTG CCTTTACCAA
1501 GCAGCTGAGG GTATGTGATA GGCAAATGTT CAGGGGCCAC TGCATGGTTT CGAATAGAAA
1561 GAGAAGCTTA GCCAAGAACA ATAGCCGATA AAGATAGCCT CATTAAACGA AATGAGCTAG
1621 TAGGCAAAGT CAGCGAATGT GTATATATAA AGGTTCGAGG TCCGTGCCTC CCTCATGCTC
1681 TCCCCATCTA CTCATCAACT CAGATCCTCC AGGAGACTTG TACACCATCT TTTGAGGCAC
1741 AGAAACCCAA TAGTCAACCG CGGACTGCGC ATCATG

FIG.18B(Cont.)

```
                10        20        30        40        50        60
                |         |         |         |         |         |
   1 CAATTCTCAC GGTGAATGTA GGCCTTTTGT AGGGTAGGAA TTGTCACTCA AGCACCCCCA
  61 ACCTCCATTA CGCCTCCCCC ATAGAGTTCC CAATCAGTGA GTCATGGCAC TGTTCTCAAA
 121 TAGATTGGGG AGAAGTTGAC TTCCGCCCAG AGCTGAAGGT CGCACAACCG CATGATATAG
 181 GGTCGGCAAC GGCAAAAAAG CACGTGGCTC ACCGAAAAGC AAGATGTTTG CGATCTAACA
 241 TCCAGGAACC TGGATACATC CATCATCACG CACGACCACT TTGATCTGCT GGTAAACTCG
 301 TATTCGCCCT AAACCGAAGT GCGTGGTAAA TCTACACGTG GGCCCCTTTC GGTATACTGC
 361 GTGTGTCTTC TCTAGGTGCA TTCTTTCCTT CCTCTAGTGT TGAATTGTTT GTGTTGGGAG
 421 TCCGAGCTGT AACTACCTCT GAATCTCTGG AGAATGGTGG ACTAACGACT ACCGTGCACC
 481 TGCATCATGT ATATAATAGT GATCCTGAGA AGGGGGGTTT GGAGCAATGT GGGACTTTGA
 541 TGGTCATCAA ACAAAGAACG AAGACGCCTC TTTTGCAAAG TTTTGTTTCG GCTACGGTGA
 601 AGAACTGGAT ACTTGTTGTG TCTTCTGTGT ATTTTTGTGG CAACAAGAGG CCAGAGACAA
 661 TCTATTCAAA CACCAAGCTT GCTCTTTTGA GCTACAAGAA CCTTCTAAAT ATATATCTAG
 721 TGGCCAGAAT GCCTAGGTCA CCTCTAAATG TGTAATTTGC CTGCTTGACC GATCTAAACT
 781 GTTCGAAGCC CGAATGTAGG ATTGTTATCC GAACTCTGCT CGTAGAGGCA TGTTGTGAAT
 841 CTGTGTCGGG CAGGACACGC CTCGAAGGTT CACGGCAAGG GAAACCACCG ATAGCAGTGT
 901 CTAGTAGCAA CCTGTAAAGC CGCAATGCAG CATCACTGGA AAATACAAAC CAATGGCTAA
 961 AAGTACATAA GTTAATGCCT AAAGAAGTCA TATACCAGCG GCTAATAATT GTACAATCAA
1021 GTGGCTAAAC GTACCGTAAT TTGCCAACGC GTTTCTAGAT TGCAGAAGCA CGGCAAAGCC
1081 CACTTACCCA CGTTTGTTTC TTCACTCAGT CCAATCTCAG CTGGTGATCC CCCAATTGGG
1141 TCGCTTGTTT GTTCCGGTGA AGTGAAAGAA GACAGAGGTA AGAATGTCTG ACTCGGAGCG
1201 TTTTGCATAC AACCAAGGGC AGTGATGGAA GACAGTGAAA TGTTGACATT CAAGGAGTAT
1261 TTAGCCAGGG ATGCTTGAGT GTATCGTGTA AGGAGGTTTG TCTGCCGATA CGACGAATAC
```

FIG.18C

1321 TGTATAGTCA CTTCTGATGA AGTGGTCCAT ATTGAAATGT AAGTCGGCAC TGAACAGGCA
1381 AAAGATTGAG TTGAAACTGC CTAAGATCTC GGGCCCTCGG GCTTCGGCTT TGGGTGTACA
1441 TGTTTGTGCT CCGGGCAAAT GCAAAGTGTG GTAGGATCGA CACACTGCTG CCTTTACCAA
1501 GCAGCTGAGG GTATGTGATA GGCAAATGTT CAGGGGCCAC TGCATGGTTT CGAATAGAAA
1561 GAGAAGCTTA GCCAAGAACA ATAGCCGATA AAGATAGCCT CATTAAACGA AATGAGCTAG
1621 TAGGCAAAGT CAGCGAATGT GTATATATAA AGGTTCGAGG TCCGTGCCTC CCTCATGCTC
1681 TCCCCATCTA CTCATCAACT CAGATCCTCC AGGAGACTTG TACACCATCT TTTGAGGCAC
1741 AGAAACCCAA TAGTCAACCG CGGACTGCGC ATG[ATG]

FIG.18C(Cont.)

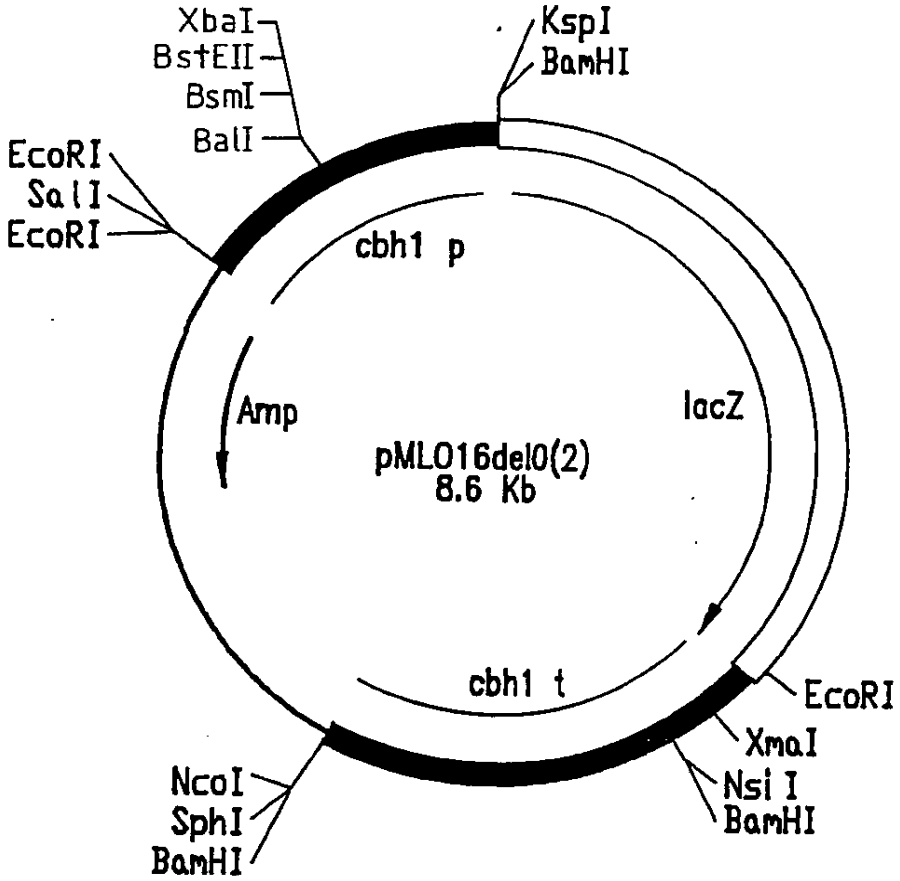
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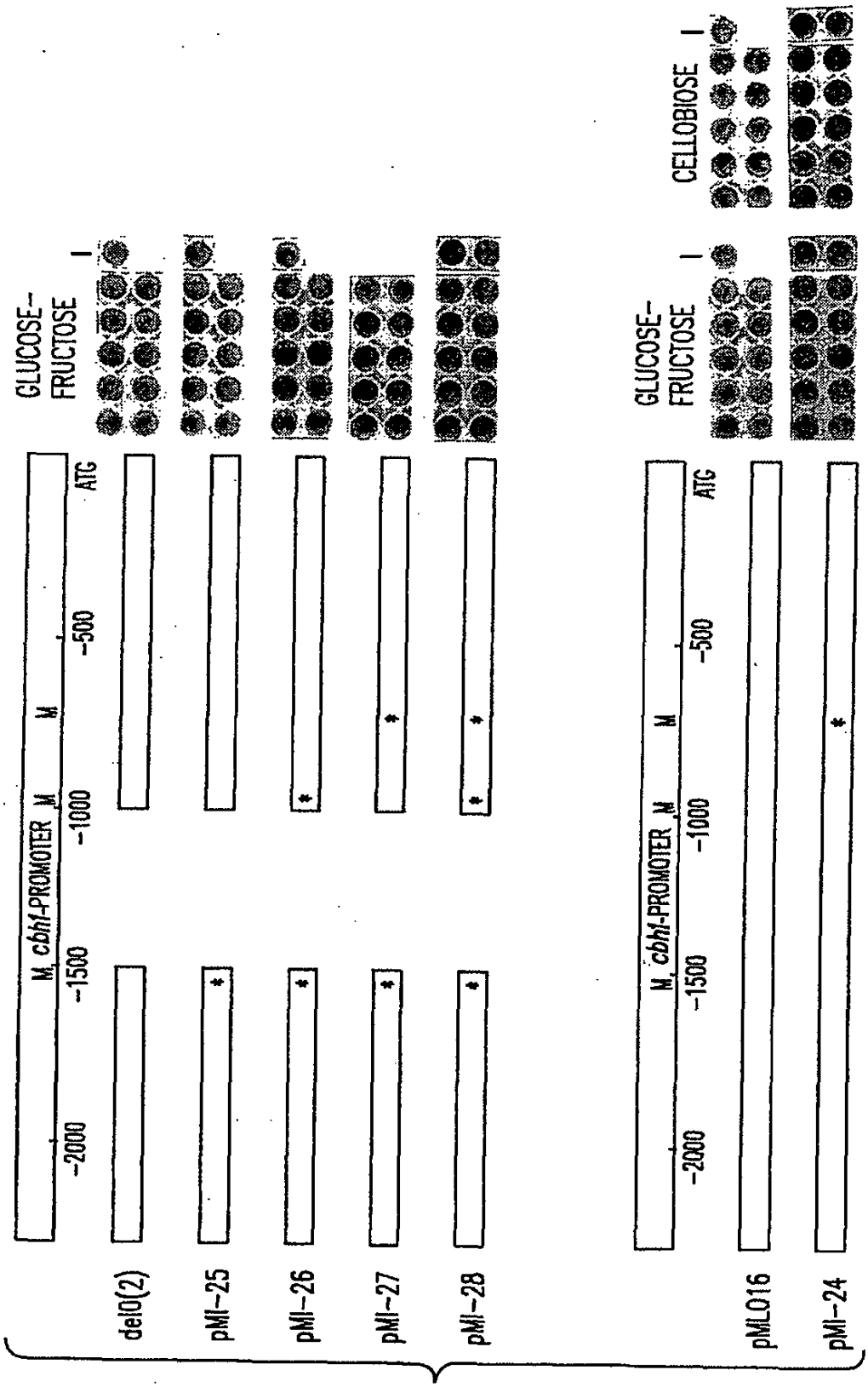
FIG.19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GLUCOSE-FRUCTOSE
CELLOBIOSE
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pMI-24

FIG.20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 93/00330

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C12N 15/11, C12N 15/56, C07K 15/04, C12N 9/42 // (C 12 N 15/11, C 12 R 1:885)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C12N, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, CA, WPI, CLAIMS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOTECHNOLOGY, Volume 7, June 1989, A. Harkki et al, "A novel fungal expression system: secretion of active calf chymosin from the filamentous fungsu trichoderma reesei", page 596 - page 603, see page 596, column 1, line 22 - column 2, line 31, page 599, column 1, lines 44-49 and the whole document --	1-40
X	EP, A1, 0137280 (CETUS CORPORATION), 17 April 1985 (17.04.85), see page 5, lines 9-24, table 1, page 30-44 and the whole document --	1-40

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 January 1994	17 -01- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Jonny Brun Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 93/00330

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information, Services, file 357, Dialog acc.no. 016146, DBA acc.no. 83-10126, Teeri T. et al: "The molecular cloning of the major cellulase gene from Trichoderma reesei - cellobiohydrolase I gene isolation cloning and characterization", Bio/Technology (1, 8, 696-99) 1983	1-6
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A	US, A, 5108918 (MARTIEN A.M. GROENEN ET AL), 28 April 1992 (28.04.92), see column 1, lines 1-68, column 4, lines 13-22, column 11, lines 46-61 and the whole document	14-20
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 93/00330

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 41
because they relate to subject matter not required to be searched by this Authority, namely:

The claim is not clear and concise and consequently it does not permit a meaningful search. (See art. 6).

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See next sheet!

1. [X] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

1. Claims 1-6: A method for cloning a promoter that is active in a desired environmental condition.

2. Claims 14-20 completely, claims 7-13 and 29-40 partially: The tef 1 promoter of trichoderma reesei and variants thereof as well as vectors and host cells comprising the promoter.

3. Claims 21-28 completely, claims 7, 13 and 29-40 partially: The cbh1 promoter of trichoderma reesei and variants thereof as well as vectors and host cells comprising the promoter.

The special technical feature of group 1 relates to a method for cloning a promoter. The method is not restricted to certain organisms or genes.

The special technical features of group 2 and 3 relate to some promoters from Trichoderma.

Methods for finding promoter sequences are well-known in the art. Hence, group 1 and the groups 2 and 3 are not so linked as to form a single inventive concept.

Trichoderma promoter sequences capable of expression of an operably-linked coding sequence in a fungal host grown on glucose are known in the art, for instance by EP-A1-137 280 or Teeri et al, Bio/technology, vol. 1, page 696-699. Consequently, the common feature (trichoderma promoter sequences) is not a special technical feature within the meaning of PCT, Rule 13.2 second sentence, since it makes no contribution over the prior art.

Therefor, there is no other feature common to claims 7-40. Since there exists no other common feature which can be considered as a special technical feature within the meaning of PCT rule 13.2, no technical relationship within the meaning of PCT rule 13 between the different inventions can be seen.

Consequently it appears that, a posteriori claims 7-40 do not satisfy the requirement of unity of invention.

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/11/93

International application No.
PCT/FI 93/00330

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0137280	17/04/85	SE-T3- 0137280	
		AU-B- 589112	05/10/89
		AU-A- 3253084	07/03/85
		DE-A- 3485558	16/04/92
		JP-A- 60149387	06/08/85
US-A- 5108918	28/04/92	AU-B- 631371	26/11/92
		AU-B- 631806	10/12/92
		AU-A- 3956889	15/02/90
		AU-A- 3956989	15/02/90
		EP-A- 0354624	14/02/90
		JP-A- 2167078	27/06/90